An Evolutionary Investigation of the New Zealand Inuleae (Compositae): Stem Anatomy and Flowering Phenology.

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"A work such as this is never really finished,
one must simply declare it finished when one has,
within the limits of time and circumstances,
done what is possible."
J.W. von Goethe

ABSTRACT

Evidence for systematic relationships and evolutionary processes in the New Zealand Inuleae (Compositae) is examined, by investigating the stem anatomy and flowering phenology of selected, representative species.

The stem anatomy of 51 species of Inuleae from New Zealand and Tasmania is described using transverse sections of the primary stem near the apex, mature primary stem, and mature secondary stem. Results from the stem anatomy provide a number of features which are available for systematic interpretation, including the occurrence of anomalous secondary growth in three species of *Raoulia*, the presence of resin canals in *Haastia*, and the occurrence of a Casparian strip. The groupings suggested by phenetic and cladistic analyses of the stem data support affinities that have already been identified in the literature, but also suggest new groupings which should be investigated.

The flowering phenology of 16 species which occur in the Cass District are described at the association, population, individual, capitulum, and floret levels. Patterns are discussed with reference to observations on floral visitors, breeding systems and habitat. It is suggested that a highly staggered flowering pattern observed in the species growing on the riverbed may have resulted from selection imposed by the occurrence and timing of floods and pollinator competition via interspecific pollen transfer. Hypotheses as to the functional significance of the phenology patterns are presented, including adaptations to avoid geitonogamy and interference between male and female functions, adaptations to specific pollinators, and opportunistic life styles. It is hypothesised that the short life span of trinucleate pollen creates a hereto unrecognised phylogenetic constraint on the evolution of the Compositae capitulum.

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1. General Introduction

The Compositae is the largest dicotyledon family (Webb *et al.*, 1990), containing some 25 000 species which have traditionally been grouped into 13 tribes (Turner, 1977). The Inuleae, as delimited by Merxmüller *et al.* (1977), is perhaps the largest of these tribes containing approximately 180 genera and some 2100 species (Merxmüller *et al.*, 1977). In New Zealand the Inuleae is the second largest tribe of the Compositae, being represented by over 60 indigenous species in 11 genera which all belong to the subtribe Gnaphaliinae (*sensu* Merxmüller *et al.*).

The largest of these genera in terms of the number of species in New Zealand, *Raoulia* Hook.f., is endemic to New Zealand and contains 23 described and at least three undescribed species (Ward, 1982). These species, which include one of the well known vegetable sheep (*R. eximia* Hook.f.) and the scabweed of Central Otago (*R. australis* Hook.f.), are split into three subgenera; *R.* subg. *Raoulia,* R. subg. *Mistura*, and *R.* subg. *Psychrophyton.*

The cosmopolitan genus *Gnaphalium* L. has the second largest representation in the New Zealand Inuleae, containing 14 species all in section *Euchiton.* Seven of these species are endemic, while the remaining seven species are shared with Australia. One species, *G. involucratum* G.Forst., is also found in Taiwan, Java, and the Philippines (Webb *et al.*, 1988).

Helichrysum Mill. is also a widespread genus, with some 500 species world wide (Merxmüller *et al.*, 1977; Webb *et al.*, 1988). The New Zealand *Helichrysum* species, which are all endemic, can be divided into two groups, the seven woody species, and three herbaceous species, *H. alpinum* Cockayne (Ward *et al.,* 1997b), *H. bellidioides* (G.Forst.) Willd. and *H. filicaule* Hook.f.

Leucogenes Beauverd and *Anaphalis* DC are each represented by four species in the New Zealand flora. *Leucogenes* is an endemic alpine genus, and is perhaps the best known of the New Zealand Inuleae with the species being known colloquially as the New Zealand edelweiss. The four indigenous species of *Anaphalis* are endemic, with other species occurring in Asia and New Guinea.

Haastia Hook.f., with three endemic species, is the only other genus in the New Zealand Inuleae (except *Craspedia* G.Forst.) represented by more than one species. Where they occur in the alpine flora, the three species of *Haastia* are a prominent feature, particularly *H. pulvinaris* Hook.f., which has also the colloquial name "(giant) vegetable sheep". *Craspedia* contains a number of endemic species, but it is more closely related to the *Angianthus* complex of Australia than to the other New Zealand Inuleae (Merxmüller *et al.*, 1977), and is therefore not considered in this thesis.

The other four genera which comprise the New Zealand Inuleae are *Pseudognaphalium* Kirp., *Ozothamnus* R.Br. (Breitwieser and Ward, 1997), *Ewartia* Beauverd, and *Rachelia* J.M.Ward et Breitw. (Ward *et al.*, 1997a). Each of these genera is currently recognised as being represented by a single species in New Zealand. *Rachelia* is a monotypic, endemic genus, whilst *Ozothamnus* and *Ewartia* also contain species in Australia. The single representative of *Pseudognaphalium*, *P. luteoalbum* (L.) Hilliard et B.L.Burtt, is a cosmopolitan species complex (Webb *et al.*, 1988).

Until recently the taxonomy of the Inuleae at the tribal and subtribal level has remained relatively stable since Bentham's treatment of the Compositae (Bentham, 1873). The only major changes have been proposed by Merxmüller *et al.* (1977) and Anderberg (1989). Under Bentham's treatment all the New Zealand taxa were placed in the subtribe Gnaphaliinae, except *Craspedia* which was placed in the Angianthinae. In the review of the Inuleae by Merxmüller *et al.* (1977) the nine subtribes recognised by Bentham in the Inuleae were reduced to three large subtribes, as a result of which all the New Zealand taxa were placed in a much more broadly defined Gnaphaliinae. As part of this review Merxmüller *et al.* (1977) also transferred the type species of *Haastia* (*H. pulvinaris*) from the Astereae to the Inuleae, suggesting that it might have affinities with the Australian genus *Pterygopappus* Hook.f. In the latest New Zealand Flora *Haastia* was transferred from the Astereae to the Inuleae (Webb *et al.*, 1988).

Cladistic analysis by Anderberg (1989) indicated that the Inuleae were not monophyletic, and consequently he divided the tribe into three. Under this system all the New Zealand taxa (except *Haastia* which Anderberg left unassigned to a tribe) were placed in the tribe Gnaphalieae, a tribe which incorporated six of Bentham's nine subtribes. At the subtribal

level Anderberg (1991b) again suggested revision, dividing the Gnaphalieae into five subtribes, and distributing the New Zealand species across four of these. Breitwieser and Ward (1993) questioned the validity of these subtribes, which separate genera they consider to be closely related.

The taxonomy at the generic level has not been so stable. This is perhaps best indicated by the high level of synonymy of the species listed in Allan (1961). *Anaphalis* and *Leucogenes*, *Ewartia sinclairii* (Hook.f.) Cheeseman, *Pseudognaphalium luteoalbum*, two species of *Helichrysum* and *Raoulia youngii* (Hook.f.) Beauverd, have all previously been included in *Gnaphalium*. *Raoulia youngii*, *Ewartia sinclairii* and *Leucogenes* have also been included in *Helichrysum*. One species of *Gnaphalium* and the three Tasmanian species of *Ewartia* have previously been included in *Raoulia*, whilst the section containing the woody species of *Helichrysum* earlier held generic status as *Ozothamnus*. The New Zealand species currently included in *Ozothamnus, O. leptophyllus* (G.Forst.) Breitw. et J.M.Ward, has previously been described as five species of *Cassinia* (see Allan, 1961), with one of these species (*Cassinia vauvilliersii* (Homb. et Jacq.) Hook.f.) also previously included in *Olearia* Moench and *Calea* L. The two main subgenera in *Raoulia* have been raised to and lowered from generic level (Beauverd, 1910; 1912). Anderberg (1991b) has proposed more changes at the generic level, shortly to be reviewed for the New Zealand taxa by Ward and Breitwieser (1998).

This instability at the generic level is perhaps best exemplified by the taxonomic history of *Helichrysum bellidioides*. Originally placed in *Xeranthemum* Tourn. ex L. by Forster (1786), *H. bellidioides* was transferred to *Helichrysum* by Willdenow (1804). Hooker transferred this species to *Gnaphalium* (Hooker, 1853), before reversing this decision 20 years later (Bentham and Hooker, 1873). Until this year *H. bellidioides* has remained in *Helichrysum*, despite suggestions that it may belong in *Anaphalioides* (Benth.) Kirp. (Drury, 1971; Webb, 1987) or in the *Lawrencella* complex of Australia (Anderberg, 1991b). A recently completed revision of *Anaphalis* in New Zealand, however, will add another generic transfer, supporting the suggestion that *H. bellidioides* belongs with four other endemic species in *Anaphalioides* (Glenny, 1997).

The difficulties at the generic level may result partially from a lack of characters which may be used to clearly delimit the generic boundaries, as many of the diagnostic characters

overlap generic boundaries (Breitwieser and Ward, 1993). Ward (1981) states that "*Raoulia* and the other genera of the New Zealand Gnaphaliinae are not clearly demarcated" and that "Many of the characters which have been used to delimit the genera have been found to over-ride generic boundaries". For example, *Gnaphalium* and *Helichrysum* have been traditionally separated by the ratio of hermaphrodite (or tubular) florets to female (or filiform) florets. However, this criterion is known to separate closely related species and to link less closely related taxa (Hilliard and Burtt, 1981). This is further complicated by *Raoulia* which occupies an intermediate position between *Gnaphalium* and *Helichrysum* in this character, a position which probably led Hooker, and later Kirk (1899), to suggest that the species now belonging to *Raoulia* subg. *Raoulia* could probably be placed in *Gnaphalium*, while those in *R.* subg. *Psychrophyton* could be placed in *Helichrysum*.

The taxonomic relationships at the generic level have been discussed recently in a series of papers that have examined the systematic relationships of the taxa in the New Zealand and closely related Tasmanian Inuleae on the basis of their morphology (Ward, 1993b; Ward, 1993a), leaf anatomy (Breitwieser, 1993), and flavonoids (Breitwieser and Ward, 1993). The results of these studies identified consistent groupings and a variable number of isolated taxa.

In all three studies (Breitwieser and Ward, 1993; Ward, 1993a; Ward, 1993b) *Raoulia* formed two main groups and a varying number of isolated taxa. The first main group included most of the species currently placed in *Raoulia* subg. *Raoulia*, except for *R. cinerea* Petrie and *R.* sp. "M" (an undescribed taxon). *Raoulia cinerea* was found to be isolated in all three studies, having weak affinities to *Raoulia*, *Gnaphalium, Ewartia* and *Helichrysum*. Ward (1993b) suggested that this species may deserve to be recognised as a monotypic genus. *Raoulia* sp. "M" clustered with the other species of *Raoulia* subg. *Raoulia* in Ward (1993a), but occupied an isolated position in Ward (1993b) and Breitwieser and Ward (1993), with some indication of affinities to *Gnaphalium* and *Raoulia*. A similar pattern occurred in the second main group formed by the species in *Raoulia* subg. *Psychrophyton*. The pulvinate species formed a consistent core within this group (Breitwieser and Ward, 1993; Ward, 1993b; Ward, 1993a), however the affinities of the four non-pulvinate species were less certain. These species showed affinities to both the pulvinate species of *Raoulia* and to *Leucogenes* (Breitwieser and Ward, 1993; Ward,

1993b). *Raoulia petriensis* Kirk, the only species in *R.* subg. *Mistura*, was found to occupy an isolated position (Breitwieser and Ward, 1993; Ward, 1993b), or to cluster with the species in *R.* subg. *Psychrophyton* (Ward, 1993a).

The species currently included in *Helichrysum* were also heterogeneous. The whipcord species formed a distinct group (Breitwieser and Ward, 1993; Ward, 1993b), to which *H. depressum* (Hook.f.) Benth. et Hook.f. showed affinities on the basis of leaf flavonoids and anatomy (Breitwieser and Ward, 1993). However, *H. depressum* had greater affinities to part of *Raoulia* on the basis of morphological characters (Ward, 1993b). The affinities of *H. filicaule* also varied, showing a strong morphological similarity to *Raoulia cinerea*, but consistently grouping with *H. bellidioides* on leaf anatomy and flavonoids (Breitwieser and Ward, 1993). Ward (1993b) and Breitwieser and Ward (1993) suggested that *H. bellidioides* and *H. filicaule* may be more appropriately placed in a genus with the New Zealand species of *Anaphalis*. The affinities of *H. lanceolatum* (Buchanan) Kirk were found to be even less clear. Breitwieser and Ward (1993) found that this species had affinities to *Ozothamnus* on the basis of its flavonoids, but that its leaf anatomy was distinct from the other taxa examined. They concluded that "Its affinities remain a mystery."

The affinities of the only New Zealand species of *Ewartia*, *E. sinclairii*, are also uncertain, as this species occupies an isolated position in both the phenetic studies to date (Breitwieser and Ward, 1993; Ward, 1993b). The affinities of the Australian species of *Ewartia* are also unclear. Ward (1993b) found that these species formed a loose cluster; however based on their leaf anatomy and flavonoids these species do not cluster closely, and show strong similarities to *Gnaphalium*, leading Breitwieser and Ward (1993) to suggest that the generic boundaries between *Gnaphalium* and *Ewartia* need revising.

Thus in these studies only *Leucogenes* and the New Zealand *Anaphalis* formed good generic groupings, while *Raoulia* and *Helichrysum* were markedly heterogeneous. A comparison of the phenograms in each of these papers (Breitwieser and Ward, 1993; Ward, 1993a; 1993b) also indicates that there is a strong suggestion of relationship between *Raoulia, Leucogenes*, *Ewartia*, and parts of *Gnaphalium* and *Helichrysum*, but that the affinities of the consistent groupings that do occur are uncertain. Thus, despite the subsequent addition of evidence from the leaf anatomy (Breitwieser, 1993) and flavonoids

(Breitwieser and Ward, 1993), the statement by Ward (1993a; 1993b), that in order to clarify and obtain a natural system of classification for the New Zealand Inuleae additional data from other fields of evidence was required, appears to still be valid.

The overall aim of this thesis was to examine two new fields of evidence, stem anatomy and flowering phenology for information that may be useful as indicators of systematic relationships or evolutionary patterns at the generic level. This study is designed to complement work in other evidential areas such as morphology (Ward, 1993a; 1993b), flavonoid analysis (Breitwieser and Ward, 1993), leaf anatomy (Breitwieser, 1993), palynology (Breitwieser and Sampson, 1997a; 1997b), molecular sequencing (Glenny, 1997; Ilse Breitwieser and Steve Wagstaff, in progress), floral micro-morphology (Lynne Baxter, in progress), and hybridism (Robert McKenzie, in progress).

With over 60 species in the New Zealand Inuleae it is clearly not feasible to examine representatives of all these species in this study. Therefore taxa were chosen for inclusion in this study to complement the studies of Ward (1993b), Breitwieser (1993) and Breitwieser and Ward (1993). These taxa were selected by these authors because they represent all the major groups in New Zealand Inuleae and their closest relatives in the Tasmanian flora. In addition, species examined for their flowering phenology were also included in the stem anatomy. In total the stem anatomy of 51 species was examined (Table 1.1).

The selection of species for the study of their flowering phenology was determined by the occurrence of species in the Cass-Craigieburn district to which regular access was available. This included two species of *Gnaphalium,* four species of *Helichrysum*, *Leucogenes grandiceps* (Hook.f.) Beauverd, *Ozothamnus leptophyllus* and nine species of *Raoulia* (Table 1.1).

Anaphalis		*A. keriensis*	
		A. rupestris	
		A. subrigida	
		A. trinervis	
Cassinia		*C. aculeata*	
		C. longifolia	
Ewartia		*E. catipes*	
		E. meredithiae	
		E. planchonii	
		E. sinclairii	
Gnaphalium		*G. audax*	(p)
		G. involucratum	
		G. nitidulum	(p)
		G. mackayi	
		G. traversii	
Haastia		*H. pulvinaris*	
		H. sinclairii	
Helichrysum		*H. bellidioides*	(p)
		H. coralloides	
		H. depressum	(p)
		H. dimorphum	
		H. filicaule	(p)
		H. intermedium	(p)
		H. lanceolatum	
		H. parvifolium	
Leucogenes		*L. grandiceps*	(p)
		L. leontopodium	
Ozothamnus		*O. leptophyllus*	(p)
		O. obcordatus	
		O. rodwayi	
Pseudognaphalium		*P. luteoalbum*	
Pterygopappus		*P. lawrencei*	
Rachelia		*R. glaria*	
Raoulia	(subg. *Mistura*)	*R. petriensis*	
	(subg. *Psychrophyton*)	*R. bryoides*	
		R. eximia	
		R. grandiflora	(p)
		R. hectorii	
		R. mammillaris	(p)
		R. subulata	
		R. youngii	
		R. sp. "L"	
	(subg. *Raoulia*)	*R. australis*	(p)
		R. cinerea	
		R. glabra	(p)
		R. haastii	(p)
		R. hookeri	(p)
		R. monroi	(p)
		R. subsericea	(p)
		R. tenuicaulis	(p)
		R. sp. "M"	

Table 1.1: Species included in this thesis for the study of their stem anatomy (all listed), and flowering phenology (p).

2. Stem Anatomy

2.1 Introduction

The anatomy of the stem provides a wide range of cell tissues and cell types which can be, and have been, used for taxonomic purposes. In the primary stem features of the pith (e.g. Carlquist, 1959c), cortex (e.g. *Casuarina* in Metcalfe and Chalk, 1950), and vascularisation have all been used systematically, while in the secondary stem the origin and structure of the periderm (e.g. Solereder, 1908) and nature of the secondary xylem have provided a wealth of additional features (e.g. Carlquist, 1988). Stem anatomy has been found to be useful for establishing or negating relationship between taxa from the species to the family level (e.g. Tippo, 1938; Hall, 1952; Erdtman and Metcalfe, 1962; Ayensu, 1970; Dickison *et al.*, 1994). The works of Solereder (1908), Metcalfe and Chalk (1950; 1983), and Carlquist (1961a; 1988) are evidence of the value of anatomy in systematic investigations.

In the Compositae a number of studies have examined aspects of the stem anatomy, both for systematic and descriptive purposes. These include the description of the vascularisation of *Helianthus* (Esau, 1945), the occurrence of anomalous secondary growth (Adamson, 1934; Moss, 1940), as well as numerous other studies listed in Metcalfe and Chalk (1950). However, perhaps the most prolific writer on the stem anatomy in the Compositae, especially in recent times, has been Sherwin Carlquist. In a series of papers Carlquist described the anatomy of the stem, node, leaf and floral structure of species in the tribes Heliantheae (Carlquist, 1957a; 1957b; 1959a; 1959c), and Mutisieae (Carlquist, 1958a). In his paper on *Dubautia, Agyroxiphium,* and *Wilkesia* Carlquist (1959c) found that the overall similarity of their anatomy confirmed the close relationship among these three genera. Furthermore, he found that features of the pith, including the type and distribution of sclereids, the thickness of the cell walls and the size of the intercellular spaces, could be used to distinguish between the species of *Dubautia.* Similarly, in *Fitchia* Carlquist (1957a) found that relationships suggested by features of the pith between *Fitchia* and other species in the *Heliantheae* were in agreement with those relationships suggested by aspects of the floral morphology. This usefulness of the stem anatomy was also repeated in his examination of nine genera in the Mutisieae (Carlquist, 1958a). In this study Carlquist concluded that the nature and distribution of sclereids in the stem and

involucral bracts, and the distribution of lacticiferous cells, provided characters which separated the genera into two closely related groups.

In a related series of papers published over a nine year period, Carlquist (1957c; 1958c; 1959d; 1960a; 1960b; 1961b; 1962; 1964; 1965a; 1965b; 1966a) described the wood anatomy of samples from all 13 of Bentham's Compositae tribes. In each of these papers Carlquist examined samples from a number of species and genera, describing the distribution and nature of the rays, vessels, growth rings, crystals and other features of the secondary xylem. In his study of the Inuleae Carlquist (1961b) included one species which is included in the present investigation, namely *Cassinia longifolia* R.Br. Amongst the features Carlquist noted for this species were the occurrence of vessels arranged in clusters, wide multiseriate rays and an absence of growth rings. The series of wood anatomy papers culminated in a paper (Carlquist, 1966b) which summarised the relationship between the wood anatomy, habit and habitat. In this paper Carlquist (1966b) states that the wood anatomy varies little from tribe to tribe, with the same basic plan present in each tribe of the Compositae. He also suggests that the most useful wood features taxonomically were those which related to habit or which occurred sporadically within the family (e.g. septate fibres). Unfortunately Carlquist (1966b) states that since the paper is primarily concerned with the relationships at the tribal level, he deliberately omitted from this paper many characters which are reliable estimators of relationship at the species and generic level. Those characters he does mention as being reliable at this level include ray structure, growth rings and crystals. In the study of the Inuleae, Carlquist (1961b) found that no one particular character of the wood anatomy separated the genera in which he had examined more than one sample. However, the combination of several characters could be used to distinguish between these genera.

Other than the study of the wood anatomy by Carlquist (1961b), three other recent taxonomic studies of the Inuleae have included characteristics of the stem anatomy. Drury and Watson (1966) included three New Zealand species (*Raoulia glabra* Hook.f., *Helichrysum lanceolatum, Ozothamnus leptophyllus*) in their brief systematic investigation of the Inuleae using comparative anatomy. They showed that the Inuleae could be split into two groups by using only a small number of anatomical characters. Three of these characters included the presence/absence of resin canals, the presence/absence of fibres within the phloem, and the diameter of the vessels in the secondary xylem. Based on the

work of Drury and Watson (1961), Anderberg (1989) also included the presence of phloem fibres and resin canals in his investigations on the phylogeny of the Inuleae, and in his study of the Gnaphalieae (Anderberg, 1991b). Unlike the third study by Puttock (1994), the papers by Drury and Watson (1966) and Anderberg provided very little detail of the stem anatomy. In his paper on *Cremnothamnus thomsonii* Puttock (1994) described and illustrated the anatomy of the annual inflorescence stems and the perennial vegetative stems. Puttock noted features such as an absence of an endodermis and resin canals, and the presence of phloem fibres, sclereids in the cortex and wide multiseriate rays. Puttock concludes that the anatomy of *C. thomsonii* is distinct from the "very few taxa (in the Inuleae) that have been currently described in detail". This paper by Puttock appears to be the most recently published work which includes detailed descriptions of the stem anatomy in the Inuleae.

In the New Zealand Inuleae the earliest publication to describe details of the stem anatomy was an examination of *Haastia pulvinaris* by Low (1899), who described the general structure of the young and old stems, as well as the general morphology of the cushion and the anatomy of the leaf. Amongst other features, she noted the presence of resin canals in the cortex opposite the vascular bundles, and the occurrence of a cork that became successively deeper with age. Although she discussed the functional significance of the leaf structure, Low offered no functional or systematic interpretation of the stem anatomy.

Hauri (1917) examined the anatomy of a number of cushion plants, including the Tasmanian peat bog species, *Pterygopappus lawrencei* Hook.f., and 13 species of *Raoulia*. From his studies Hauri concluded that the cushion plants he had examined were morphologically and anatomically xerophytic, and that the morphological convergence in the growth form was associated with an anatomical convergence. He concluded that convergent features included the occurrence of mechanical strengthening elements in the leaves, and the early and strong development of the cork in young stems. He stated that all the cushion plants he examined showed strong development of the cork, with $\frac{1}{3}$ to $\frac{1}{2}$ of the radius in the young stem being occupied by the cork. Hauri also noted the occurrence of calcium oxalate crystals in *Raoulia bryoides*, and the early lignification of the pith in all 13 species of *Raoulia*.

Foweraker (1917) studied the general morphology and anatomy of the mat and cushion forming plants growing in the vicinity of the University of Canterbury Field Station at Cass. These included *Raoulia haastii* Hook.f., *R. lutescens* (= *R. australis* Hook.f.), *R. australis* (= *R. hookeri* Allan), *R. monroi* Hook.f., *R. subsericea* Hook.f. and *R. tenuicaulis* Hook.f. In his discussion on the anatomy of *R. monroi*, Foweraker (1917) remarked on the similarity of their stem anatomy, stating,

> "Transverse sections of the young stem show the same appearance as in the other species of *Raoulia*; indeed, the differences between the young stems of all species are but slight." (Foweraker, 1917, p. 31)

In the mature stems Foweraker noted differences between the species in features such as the extent of secondary growth, the number and extent of "pericyclic" fibres, and the appearance of the endodermis. Foweraker concluded that many of the features he described, including the early lignification of the pith and the occurrence of pericycle fibres, allowed them both to withstand crushing and to force their stems through the substrate.

A feature in which Foweraker (1917) took a particular interest was the endodermis, stating that "the most striking feature in the stem-anatomy of the raoulias is the well-developed endodermis." He concluded that the strongly developed endodermis must be "of considerable importance in relation to edaphic conditions". Citing Haberlandt (1914), Foweraker suggested that the well developed endodermis may isolate the central part of the stem from the cortex, thus allowing "considerable negative pressures to be maintained in the water conducting channels", thereby allowing the plant to withstand alternate periods of abundant water-supply and severe drought.

Betts (1920a; 1920b) gave detailed descriptions of the stem anatomy of *Gnaphalium traversii* Hook.f. and *Cassinia vauvilliersii* var *rubra* (now included in *Ozothamnus leptophyllus* Breitw. et J.M.Ward). In *G. traversii* she noted the occurrence of a well marked endodermis and the presence of large thin walled cortex cells which she interpreted as water storage tissue. In *O. leptophyllus* Betts noted, amongst other features, large groups of pericyclic fibres and an apparent absence of rays.

Neither Foweraker (1917), Hauri (1917), nor Betts (1920a; 1920b) offered any systematic interpretations for the stem anatomy of the species they examined.

Two recent studies have included stem anatomy in investigations attempting to verify the taxonomic status of putative hybrids between species in the New Zealand Inuleae (Jordan, 1995; Falvey, 1996). Jordan (1995) examined samples of *Raoulia glabra, Helichrysum filicaule, H. bellidioides,* and *H. lanceolatum* as potential parental species of *H. purdiei* Petrie, including features of the pith and endodermis and the amount of secondary xylem. Falvey (1996) included features of the pith and cortex and the amount of secondary growth in her examination of three hybrids which she resolved to be the result of hybridisation events between *R. mammillaris, Leucogenes grandiceps*, and *H. bellidioides*. Falvey (1996) also noted the occurrence of a non-cylindrical stem shape in the mature axis of *R. mammillaris*.

Todd (1996), in a taxonomic investigation of *Haastia*, examined the stem and leaf anatomy of *H. sinclairii* Hook.f., *H. recurva* Hook.f. and *H. pulvinaris*. However, he included only one character of the stem anatomy, the amount of secondary thickening, in his results and phenetic analysis, and did not provide descriptions of the anatomy of the three species.

Thus, despite early and continued interest, the stem anatomy for most of the species in the New Zealand Inuleae has not been examined. Furthermore, many of the studies which provided detailed descriptions of the stem anatomy were either purely descriptive or included only functional interpretations. Given the utility of stem anatomy in elucidating systematic relationships in other groups, it might also be expected to be useful in this respect in the New Zealand Inuleae. The aim of this study was therefore to search for characters which would shed further light on the systematic relationships within the New Zealand Inuleae and with related Tasmanian Inuleae, by examining the stem anatomy of a representative sample of taxa using transverse sections.

2.2 Materials and Methods

2.2.1 Materials

Fresh plant material was collected in the field from healthy plants between October and April, on trips in the South Island, New Zealand. All material was fixed in formalin acetic alcohol (FAA) as soon as possible after collection and vacuumed overnight to remove any air pockets. After approximately two months the FAA was replaced with 70% ethanol for final storage. Material of Tasmanian and North Island taxa was obtained from collections made by Dr. J.M. Ward. Species examined are listed in Appendix 1.

Two collections of each species were examined whenever material was available. The species for which only one collection was examined were *Anaphalis subrigida* (Colenso) C.Webb, *A. rupestris* C.Webb, *A. trinervis* (G.Forst) F.Muell., *A. keriensis* (A.Cunn.) C.Webb, *Leucogenes leontopodium* (Hook.f.) Beauverd, and *Rachelia glaria* J.M.Ward et. Breitw. For each specimen the following three stages of stem development were examined from healthy vegetatively growing stem:

A. Young stem immediately behind the growing tip,

B. Mature stem still in the first year of growth that had not yet commenced secondary growth,

C. Mature stem showing secondary growth, preferably two or more years of age.

The only exceptions were the rosette species *Gnaphalium audax* D.G.Drury, *G. involucratum*, and *G. traversii*. For these species material from the rosette and the stolon was examined. The sections from the three stages of stem development will be referred to as tip sections (A), mature primary stem sections (B), and mature secondary stem sections (C). The last two type of sections will be abbreviated to MPS and MSS sections, respectively.

Herbarium vouchers for all plants examined are deposited in the University of Canterbury Herbarium (CANU). Collection data are given in Appendix 1.

2.2.2 Methods

A standard alcohol series was used for all techniques, involving single changes of 30, 50, 70, 85, 90, 95% ethanol and two changes of 100% ethanol. All changes were a minimum of 20 minutes for small pieces and two or more hours for larger pieces. If material was to

be mounted in Depex, the alcohol series was extended to include a single change of 50/50 ethanol (100%) to xylol, and two changes of 100% xylol.

Resin Sections

Pieces of preserved stem 3-5 mm in length were cut for each of the three stages (tip, MPS and MSS). These were vacuumed in 70% ethanol overnight before being taken up an alcohol series into 100% ethanol and transferred into Technovit infiltrating solution (1 g of hardener I mixed with 100 ml Technovit 3040 solution from the Technovit #7100 resin kit (Kulzer, Germany)). Specimens were allowed to infiltrate at 4°C for a minimum of 14 days, before being embedded in 2 ml EMS polythene capsules. Specimens were embedded by half filling the capsules with Technovit embedding resin (a 1:15 mix of hardener II and infiltrating solution), before the specimens were introduced and oriented. Backing resin (Technovit 3010) was applied when the embedding resin had almost set completely except for a thin surface layer. Specimens which were too large to embed in capsules were embedded in inverted capsule lids. These blocks were backed by placing the righted lids on gelatine capsules filled to overflowing with backing resin. Prior to sectioning, blocks made in capsule lids were trimmed to provide as small a cutting face as possible. Transverse sections of 5µm thickness were cut on a Jung rotary microtome equipped with glass knives made on a LKB 2078 Histo Knife maker. Ten to 20 sections were placed serially in water drops on each slide, then the slides dried on a dish warmer set to 60°C. Sections were stained with 1% aqueous Azur II (Gurr - Certistain) by immersing the slides in stain for 10 seconds. Slides were then rinsed in a series of water baths until the water no longer became coloured by the stain. The slides were then air dried, before being permanently mounted using Depex.

A trial to evaluate the best staining method was conducted using sections cut from blocks of tip, MPS and MSS of *Helichrysum intermedium* G.Simpson and *Raoulia subsericea*, prepared and sectioned as described above. Slides from each of these blocks were stained for intervals ranging from 10 to 30 seconds, with 1% aqueous Methylene Blue (Gurr - Certistain), or 1% aqueous Azur II, or Methylene Blue followed by Azur II. This trial showed that Methylene Blue and Methylene Blue plus Azur II consistently overstained the sections, even with staining times of 10 seconds. By comparison, a staining time of 10 to 15 seconds with Azur II produced results with good clarity and contrast, in which lignified or suberised cell walls stained a pale blue, and cellulose cell walls stained dark blue.

Sledge Sections

In addition to resin sections, sledge sections were cut for *Helichrysum parvifolium, H. lanceolatum, H. coralloides, Ewartia sinclairii,* and *Raoulia eximia.* Preserved stem specimens of approximately 1-1.5cm length were transferred from 70% to 50% ethanol, and then into distilled water, before being placed under vacuum for 24 hours. Specimens were then transferred into 50% ethanol until they were sectioned. Transverse sections of 30 or 40 μm were cut using a sledge microtome fitted with a metal blade, then transferred into 50% ethanol using a brush. Sections were prepared for mounting by staining with 1% aqueous Safranine O (Gurr - Certistain) and 1% Fast Green FCF (Gurr - Certistain) in 95% ethanol as part of the standard alcohol series. Staining times were 15 minutes and 30 seconds for Safranine and Fast Green respectively. The alcohol series was continued into xylol, before sections were permanently mounted in Depex.

Scanning Electron Microscope

Roughly trimmed specimens were transferred from 70% into 50% ethanol for two to three days before the specimens were trimmed to size. Each block was cut using a new razor blade to expose the desired surface. The specimens were then allowed to air dry for a minimum of five days. Once dry the specimens were mounted on an aluminium stub and coated with gold using a Polaron E5000 splutter coater. All specimens were viewed using a Leica S440 Scanning Electron Microscope fitted with an Oxford Energy Dispersion Spectra Analysis unit.

Nomenclature

The names used in this study follow Allan (1961), Webb (1987; 1988), Molloy (1995), Ward *et al.* (1997a), and Breitwieser and Ward (1997) for the New Zealand taxa, and Curtis (1963) and Wilson *et al.* (1992) for the Tasmanian taxa. A list of species names used is given in Appendix 1.

In addition to the formal nomenclature, the term "woody" *Helichrysum* is used to refer to *H. coralloides* (Hook.f.) Benth. et Hook.f., *H. depressum, H. dimorphum* Cockayne, *H. intermedium, H. lanceolatum,* and *H. parvifolium* Yeo. The term "whipcord" *Helichrysum* is used to refer to *H. coralloides, H. intermedium,* and *H. parvifolium*. The term "pulvinate" *Raoulia* is used to refer to four species of *Raoulia* subg. *Psychrophyton*

included in this study, *R. bryoides* Hook.f., *R. eximia*, *R. mammillaris* Hook.f. and *R.* sp. "L".

Miscellaneous

Sections were examined using an Olympus CH2 compound microscope fitted with a polarising filter. Photographs were taken on an Olympus BH2 fitted with an Olympus photographic unit, using Ilford Pan F Plus black and white film and Velvia Colour slide film.

Hand sections of preserved stem material of some species were cut with razor blades and stained with aniline sulfate to check determination of lignified tissue. These preparations were viewed as temporary aqueous mounts.

Measurement of the diameter of tissues in mature sections was performed using Metamorph™. A digital image was captured from a video camera attached to an Olympus BH2. The image was then calibrated and the desired measurements recorded. These included measurements of pith diameter, stem radius, maximum vessel diameter, cortex radius, and xylem radius.

Unless indicated in the text the terms used follow those listed by IAWA Committee on Nomenclature (1957; 1964) or Esau (1953). The term "consistent" is used to indicate the occurrence of a feature in both samples which were examined for each species. "Inconsistent" is used to indicate the presence of a particular character state in only one of the samples examined. The phrase "young stem sections" is used to refer to tip and mature primary stem (MPS sections).

The term (leaf) "sheath" is used to refer to the region of the petiole immediately above the separation of the leaf from the stem

All plates are from transverse resin sections unless otherwise indicated.

Character selection

Characters were selected following an initial survey of approximately half the taxa representing all the major groups (i.e. genera, sections, subgenera). Characters chosen for

the complete survey were selected for ease and accuracy of consistent identification from transverse sections.

Cladistic Analysis

An initial data matrix of 95 samples by 103 characters was obtained. Characters that showed no consistency within a specimen were eliminated. The two specimens of each species were then combined into a single evolutionary unit (EU), resulting in a data matrix of 36 characters by 51 EUs. All characters were coded as unordered. The states for each character are listed in Appendix 2.

This data matrix was analysed using PAUP (Swofford, 1991) with the following settings: MAXTREE = 3000, MULTISTATE = polymorphism, COLLAPSE = yes, BRANCH SWAPPING = TBR. Five analyses runs were performed using Heuristic search methods initiated using a random addition sequence. The initial random seed was generated using the rand() function in Microsoft Excel. At the end of each search all trees and consensus trees were rooted at *Cassinia aculeata* R.Br. and *C. longifolia*, and saved to file. (This rooting position was chosen so as to present the cladograms in an orientation consistent with those of Breitwieser (1990).) A sixth analysis was also run, for which all settings were the same as above, except that MAXTREE was set to 10 000. (This represented the upper limit that the computer was able to search.) Trees were drawn in MacClade (Maddison and Maddison, 1992).

Numerical Methods

The character matrix used in the cladistic analysis was also analysed using cluster analysis and principal coordinate analysis (PCoA). In addition to the characters included in the cladistic data matrix the following quantitative characters were included: the maximum stem radius, the maximum vessel diameter, and the proportion of stem radius occupied by the pith, xylem and cortex.

The cladistic data set included some taxa with variable states for a single character. The phenetic and PCoA methods available were unable to deal with these "multistate" taxa. This problem can be solved either by redefining the character states, or by splitting the characters in which the multiple states occur for one taxon into a series of binary characters. The process of redefining the character states was not used, since this could

potentially mean that any differences in the results obtained from different analysis methods may result from different data sets, rather than from different analysis techniques. All characters that contained taxa with multiple states were therefore re-coded into a series of binary characters. However, re-coding a single character into a series of binary characters in this way introduces a form of character weighting. While it is accepted that character weighting is implicit in all taxonomic studies through choice of taxa, characters and character states etc, it was believed to be desirable to have all characters submitted to the different analyses with equivalent weightings. This necessitated the inclusion of a fourth similarity coefficient, the "stepped coefficient", to the three already used by the Gower's General coefficient of similarity (see below). The stepped coefficient and the problem of character weighting are discussed further in Appendix 5.

The similarity matrix for the PCoA and the phenetic analysis was generated using Gower's General similarity coefficient, which allows the use of quantitative, qualitative and dichotomous characters (see Appendix 5). This matrix was created using an algorithm written and run in S-Plus for Windows. Average, also called UPGMA, and single linkage clustering was done using the hclust function in S-Plus, and dendrograms were produced using the plclust command. The cophenetic correlation coefficient was calculated in an algorithm written for S-Plus which utilised the cor.test function. This algorithm allowed the cophenetic correlation to be calculated for the whole dendrogram or to be measured after the fusion of each OTU or cluster of OTUs.
PCoA was done in S-Plus using the cmdscale function. Scatter plots were created in S-Plus using the eqscplot function from the mass library of Venables (1994).

2.3 Results

General Description of the Stem Anatomy: In the young stem the pith is normally composed of thin-walled parenchymatous cells (e.g. Plate 1E). The pith is surrounded by a ring of collateral bundles, which give rise to numerous leaf traces. The xylem is formed by a mix of thickened primary elements and parenchymatous cells (e.g. Plate 2D). The stele (i.e. the vascular tissue, the ground tissue between the vascular bundles and the pith) is surrounded by an endodermis, which may be more or less conspicuous in the young stem. The endodermis appears to undergo periclinal divisions in the very young stem near the

stem apex, sometimes making it difficult to detect in the youngest sections. The cortex is composed of generally thin-walled parenchymatous cells with intercellular spacing of varying extents and distribution (e.g. Plate 8A, B). The cortex cells in all species appear to show collenchymatous type thickenings, especially in the region of leaf sheath development. The cortex cells of many species contain chloroplasts, especially the outermost cells (e.g. Plate 8E). The stem is bounded by an epidermis of a single layer of cells, and may contain stomata in some species (e.g. Plate 9D, E). A dense layer of uniseriate and/or biseriate hairs may also be present.

In the mature stem the arrangement of the tissues remain approximately the same, modified only by the lignification of cells and development of a periderm in some species. The pith and some cortex cells of most species become thickened and lignified in the mature stem (e.g. Plate 1A, C; Plate 9A, B, C). Secondary phloem and xylem are produced by a single vascular cambium that develops between the primary xylem and phloem. The secondary xylem in all species is composed entirely of lignified elements. Growth rings occur in the secondary xylem of some species. In the pericyclic region of nearly all species, sclerenchymatous fibres develop (e.g. Plate 5D, E). These normally develop opposite the primary vascular bundles, but may extend throughout the entire pericycle region of some species.

In the mature stem the endodermis is normally conspicuous due to thickenings of the cell walls (e.g. Plate 7A). In some species the endodermis or epidermis may form the outer layer in the mature stem (e.g. Plate 12A). In most species, however, the development of a periderm in the outer cortex or in the pericyclic region of the phloem results in the loss of the epidermis, the cortex, and often the endodermis (e.g. Plate 12C). The periderm is generally composed of large pale staining cells, indicating the presence of suberin and/or lignin. The periderm initially appears to develop in the cortex, and in some species remains superficial. In the species with greater secondary growth the periderm gradually becomes more deeply seated, so that in the fully mature stem it is located in the outer region of the phloem. In all species which were examined, the phellogen appears to produce cells only to the outside.

2.3.1 Character description and distribution

The following section provides descriptions of the characters and provides examples of the distribution of the character states that were observed. A complete list of characters and character states and a table of character states for each species are provided in Appendices 2 and 3 respectively. Characters which had a high level of inconsistency or uncertainty are included below for completeness, but were not used in the analyses. These characters are indicated with the abbreviations "inc" or "unc" after the character name.

Pith end walls: The appearance of the end walls of the pith cells was clearly visible in some of the transverse sections of each specimen. In the tip sections the end walls appeared to be either fibrous, grainy, or smooth. In young stems the appearance of the pith end walls was inconsistent. By contrast, in mature stems the appearance of the pith cell end walls was consistent for any given species. In all species, except five, the end walls of the mature stem had a smooth appearance, which was interrupted by a variable number of primary pit fields (Plate 1A). Of the five exceptions, four were species of *Gnaphalium* and the fifth was *Ozothamnus leptophyllus*. The four *Gnaphalium* species had an end wall that generally appeared smooth, but had a grainy texture, while the pith end walls of *O. leptophyllus* had a fibrous appearance (Plate 1B).

Thickening of the pith cell walls: The cell walls of the pith were observed to vary in the extent and type of thickening.

In young stems all species, except *Haastia pulvinaris*, had a pith composed of cells with unthickened walls, or cells with only slight collenchymatous type thickenings at the corners of the cells. As the stems aged the extent of the wall thickenings generally increased, and the walls often became lignified. In *H. pulvinaris* the pith walls were found to be lignified and thickened in all stages and samples of stem examined.

The collenchymatous type thickenings which were observed could be divided into two types. In most species uneven thickening developed at the corners of the cells (e.g. Plate 1E) and gradually extended around the entire cell walls as the stem aged (Plate 1F). The cell walls with this type of thickening frequently became lignified in the MSS sections, once the entire cell walls had become evenly thickened. The second type of

collenchymatous thickening was characteristic of *Gnaphalium traversii* and *G. audax*. In both these species the pith walls in the rosette had a warty appearance, caused by numerous small thickenings at intervals around the pith cell walls (Plate 1D).
Lignified pith cell walls were observed to vary from moderately thickened (e.g. *Leucogenes grandiceps*, Plate 1C, most cells) to very prominently thickened (e.g. *Helichrysum coralloides*, Plate 1A). Generally the lignified cell walls were of an even thickness both within a cell and among pith cells of the same species. However, in one specimen of *L. grandiceps* a few pith cells developed extremely thick cell walls (Plate 1C).

In a few species the walls of the cells at the periphery of the pith were observed to be lignified, while the central pith cells were unlignified, having collenchymatous type thickenings. This suggests that lignification of the pith proceeds from the periphery to the centre.

In the MSS sections pith cells with no thickenings were found consistently only in *Gnaphalium involucratum*. *Ozothamnus leptophyllus* was the only species in which the pith of the MSS were observed to have collenchymatous thickenings only; all other species had a pith composed of lignified or lignified and collenchymatous cells.

Intercellular pith spaces: The intercellular spaces between pith cells were found to be filled to varying extents by the middle lamella. The extent to which intercellular spaces were filled was divided into three categories; unfilled (Plate 2A), partially filled (Plate 2B), and completely filled (Plate 2C). Many specimens showed a combination of unfilled and partially filled, or partially filled and completely filled intercellular pith spaces. In tip sections intercellular spaces that appeared unfilled or partially filled were observed in over half the species examined. As the stem matured, however, the intercellular spaces became increasingly filled. In the mature secondary stem sections only six species consistently had partially filled intercellular pith spaces (*Anaphalis rupestris, A. trinervis, Gnaphalium audax, Helichrysum depressum, H. filicaule*, *Raoulia hectorii* Hook.f.), and no species were observed to have consistently unfilled intercellular spaces between pith cells.

The middle lamella filling the intercellular spaces also changed in nature as the stem matured. In tip sections of all species, except those of *Haastia pulvinaris*, the middle

lamella was stained dark blue. In all sections of *H. pulvinaris* the middle lamella was found to stain pale blue, indicating that it had become lignified. In other species where the middle lamella became lignified this only occurred in the mature stems. In the mature secondary stem sections the middle lamella of the pith consistently remained unlignified in only nine species, compared to 21 species in the tip sections.

Lignified primary xylem: The primary xylem of all species remained readily recognisable in the mature stems. In most species darkly stained, thin walled parenchymatous cells were apparent (Plate 2D, E). In some species, however, all the cells in the primary xylem stained pale and were strongly birefringent, indicating that they had become lignified (Plate 2F). In these species the lignification of the cells in the primary xylem tissue occurred whether or not the cell walls increased noticeably in thickness. Lignification of all primary xylem cells was most common in *Raoulia*, being observed in 11 of the 18 species examined. It was also found in *Helichrysum bellidioides*, *H. filicaule*, *Haastia sinclairii*, *Leucogenes leontopodium*, and *Pterygopappus lawrencei*.

Vessel grouping: Vessels were observed in the secondary xylem either as solitary cells, or in groups arranged in tangential, radial or clumped aggregations. Thirty-three species were found to have grouped vessels. Of these, 19 contained more than one type of vessel aggregation, and only 13 species were consistent (i.e. same type or combination of types observed in both specimens).

Tangential vessel groupings were the most commonly observed type of vessel aggregation (Plate 3A). In most species the number of vessels in each tangential group may be as few as two or three, or as many as six or more. Tangential groupings often occurred in the early wood of a growth ring, but were usually less frequent (or occasionally absent) in the late wood. Tangential vessel aggregations occurred in some species of all genera except *Gnaphalium*, *Pseudognaphalium*, and *Pterygopappus*. (The last two genera contained only solitary vessels. (Plate 3D))

Radial vessel aggregations were observed in species of all genera with grouped vessels with the exception of *Ewartia*. All radial groupings were one vessel wide tangentially, but varied in the number of vessels in radial direction. In most species the radial groupings were formed by a small number of vessels, usually two to five vessels. In *Helichrysum lanceolatum*, however, the radial aggregations frequently contained 10 or more vessels.

One such radial group of vessels contained over 39 vessels and crossed two growth rings (Plate 3B).

Clumped vessel aggregations were observed in only six species including both species of *Cassinia*, *Ozothamnus leptophyllus*, *O. rodwayi* Orchard, *E. sinclairii*, and *Haastia pulvinaris*. Clumped vessel groupings were of approximately equal width in radial and tangential directions, and normally contained eight or less vessels. Clumped aggregations were observed occasionally with a slightly diagonal appearance. In these groupings the radial dimensions tended to exceed the tangential dimensions (Plate 3C).

Some species with only solitary vessels occurred in all genera except *Cassinia* and *Ozothamnus*.

Type of rays (Unc): Information on the occurrence and type of rays is best obtained from longitudinal sections, since this allows the observation of the complete ray structure, and the examination of greater lengths of stem. Despite this, some preliminary information can be obtained from transverse sections about the distribution of multiseriate rays, but care must be used when interpreting what appears to be a rayless or uniseriate condition.

Of the species examined 13 were consistently observed to lack rays. These included six species of *Raoulia*, three species of *Gnaphalium*, *Ewartia planchonii* (Hook.f.) Beauverd, *Helichrysum filicaule, Pterygopappus lawrencei* and *Rachelia glaria.* Another 12 species were inconsistent for the occurrence of rays, with rays being observed in one of the two specimens.

In species in which rays were observed, the rays appeared to be of three types: uniseriate, multiseriate, or medullary. Medullary rays, also termed primary rays (IAWA Committee on Nomenclature 1957; 1964), were observed to be a prominent feature of wood in some species of *Raoulia, Gnaphalium* and *Anaphalis*. These rays were usually at least four cells wide and could be traced inwards to the pith (Plate 4A). The ability to trace the rays to the pith distinguished them from multiseriate rays.

Prominent multiseriate rays were observed in *Raoulia eximia* and most species of woody *Helichrysum* and *Ozothamnus*. Multiseriate rays varied from two or three cells wide (e.g. *H. lanceolatum*; Plate 4B) through to 10 or more cells wide (e.g. *Raoulia eximia*; Plate 4C).

Uniseriate rays were observed inconsistently in six species. In each of these six species multiseriate or medullary rays were observed in the second specimen. It was therefore unclear whether the "uniseriate" rays observed represent a uniseriate ray proper, or the top cells of a multiseriate ray. The preliminary SEM investigation, however, confirmed occurrence of uniseriate rays in one specimen of *Cassinia longifolia* (Plate 4D).

Parenchyma distribution in the secondary xylem (Unc): Axial parenchyma was consistently identified in the secondary xylem of only 10 species. In these species the parenchyma was associated with vessels and may therefore be termed paratracheal (Plate 4E, F).

In the remaining species the occurrence of axial parenchyma was both inconsistent and uncertain. Uncertainty in the identification of axial parenchyma was caused by the lignification and thickening of the cell walls of all xylem elements. This was compounded by the inability to observe living cell contents. (The occurrence of thinner walls and living cell contents are features listed by Carlquist (1961a) as features to use in the identification of axial parenchyma.) It is therefore unclear whether the apparent absence of axial parenchyma in many species is due to difficulty in identifying parenchyma from transverse sections, or represents a true absence. Even in many species where axial parenchyma was observed, the occurrence was found frequently to be inconsistent, occurring in only one of the two specimens.

Type of growth ring: Growth rings were observed in 22 species, including species of *Anaphalis, Helichrysum, Ozothamnus, Raoulia, Ewartia* and *Haastia*. A common feature delimiting the growth rings in all 22 species was variation in the radial width of the imperforate tracheary elements (Table 2.1, Type 1). The tracheary elements reduced in width only gradually through the growing season, but the difference in width between the late wood of one growth ring and the early wood of the next was marked. The occurrence of wider tracheary elements in the early wood was the only feature observed to delimit growth rings in 12 species. In five species growth rings were also marked by changes in

vessel diameter and/or abundance (Table 2.1, Type 2 and 3). In these species more or wider vessels were present in the early wood (e.g. *Raoulia tenuicaulis,* Plate 4A; *R. haastii*, Plate 3E). In *R. tenuicaulis*, however, the changes in vessel size varied, decreasing gradually in some rings, but increasing then decreasing in others.

In four species the arrangement of the vessels was observed to change from larger, usually tangential groups in the early wood to smaller groups or solitary vessels in the late wood (e.g. *Haastia pulvinaris,* Plate 3A). This change in grouping may possibly be due to a decrease in the abundance of vessels, rather than a change in the arrangement of the vessels *per se* (Table 2.1, Type 4).

The final variation, observed in *Helichrysum depressum*, was an increase in the abundance of thin walled imperforate trachery elements in the early wood (Plate 3F) (Table 2.1, Type 5).

	1	2	3	4	5
Imperforate cells radially wider in early wood	+	+	+	+	+
Change in vessel size		+	+	±	
Change in vessel abundance			+	±	
Change in vessel grouping				+	
More thin walled imperforate cells in early wood					+

Table 2.1: Features delimiting the five observed types of growth rings.

Maximum vessel diameter: The maximum vessel diameter, as measured tangentially including the cell walls, varied from 10 μm in *Raoulia* sp. "L" to 67 μm in *R. tenuicaulis*. Most species, however, varied between 15 and 35 μm.

The difference in maximum vessel diameter between two specimens of the same species were usually found to be less than 6 μm, and exceeded 10 μm in only seven species (*Ewartia planchonii, Ozothamnus obcordatus* D.C., *Leucogenes grandiceps, Raoulia bryoides, R. glabra, R. subsericea,* and *Helichrysum bellidioides*).

There was a significant relationship between habitat and maximum vessel diameter ($X^2 = 42.7789$, df = 16, p = 0.0003), with the species which grow in riverbed and shrub communities having a higher frequency of wide vessels than those found growing in grassland or alpine communities.

Anomalous cambium activity: Anomalous cambial activity was observed only in three species of *Raoulia*: *R. bryoides, R. eximia,* and *R. mammillaris*. This anomalous cambium activity took the form of uneven production of secondary xylem around the circumference of the stem. It is apparent from the sections that the activity of the cambium had either totally ceased in places, or at least been greatly reduced. The result of the anomalous growth was the production of large lobes of secondary xylem. In some specimens a single large lobe was produced in one particular direction (Plate 5A). In other specimens, however, the anomalous activity produced two or more lobes separated by large fissures (Plate 5B). Another feature associated with this growth pattern was the restriction of phloem fibres to areas on the lobes (Plate 5C).

The cessation of cambial activity appears to have affected individual stems at different time intervals. The cambium in one specimen appeared to have ceased functioning around most of the stem in the first or second year of growth (Plate 5B - note the persistence of leaves opposite the lobe of secondary xylem). In other specimens full cambial activity appears to have been retained for at least four or five years. The cause of the anomalous cambial activity in these three species is not apparent from the transverse sections, or preserved specimens. However, it did not appear to be associated with branching.

A number of other species with large amounts of secondary growth sometimes exhibited a tendency for more xylem to be produced on one side of the stem. In all of these species, however, the cambium remained active around the entire circumference of the stem.

Fibres in the phloem: Cells of a sclerenchymatous nature (hereafter referred to as phloem fibres) developed in the region of the outer phloem in many species as they matured. In the tip sections only the three stoloniferous *Gnaphalium* species and *Helichrysum lanceolatum* possessed phloem fibres. In the mature stems, however, nearly all species displayed phloem fibres. The only exceptions were the two alpine *Gnaphalium* species (*G. nitidulum* Hook.f. and *G. mackayi* (Buchanan) Cockayne), *Ewartia meredithiae* (F.Muell.) Beauverd

and *E. planchonii*, *Helichrysum dimorphum*, *Pterygopappus lawrencei* and three members of *Raoulia* subg. *Psychrophyton* (*R. grandiflora* Hook.f., *R. hectorii*, and *R.* sp. "L").

Two species were inconsistent for fibre presence, with well developed fibres observed in only one of the two samples. These were *Ewartia catipes* (D.C.) Beauverd and *Raoulia cinerea.*

Type of fibres: In those species that possessed phloem fibres two main arrangements could be distinguished. In most species the fibres were clearly recognisable as individual cells (even when the intercellular material was also lignified). The lumen of these fibres varied from large (e.g. *Helichrysum depressum*, *Anaphalis* species) to almost absent (e.g. *Haastia pulvinaris,* Plate 5D). In nearly all species of *Raoulia*, however, the fibres (when present) formed a large mass in which individual cells were often difficult to distinguish (Plate 5E). In addition, the lumen in these fibres was always large. In some species of *Raoulia* the fibres were initially produced in a mass, but subsequent fibres appear to be clearly separate (e.g. *R. australis,* Plate 5F). The only species outside of *Raoulia* in which the phloem fibres appeared to form a mass was one of the two specimens of *Leucogenes grandiceps*.

Casparian strip: A casparian strip (Plate 6A to F) was a conspicuous feature in the endodermis of all species of *Anaphalis* (except *A. trinervis*) and *Haastia*, and four of the six woody species of *Helichrysum* (the two exceptions were *H. lanceolatum* and *H. depressum*). The remaining species in which Casparian strips were observed were *Gnaphalium mackayi*, *G. traversii, Ewartia catipes, Raoulia cinerea*, and *R.* sp. "L". The presence of casparian strips was consistent in all species except *E. catipes* and *H. intermedium.*

In these species the casparian strip was visible as a pale blue band and thickening in the cell walls of one or both of the young sections. In both *Haastia* species the presence of the Casparian strip was the only feature which distinguished the endodermis cells from those of the cortex. The endodermis cells also formed part of the ring of cells enclosing the resin canals (Plate 6D).

Endodermis walls birefringent: When examined under polarised light the endodermis walls of some species were found to be strongly birefringent (Plate 7A, B). The endodermis walls that exhibited strong birefringence were all stained pale indicating the presence of lignin and/or suberin.

In many of the species in which these strongly birefringent endodermis walls were observed, the cell walls were thickened. However, not all species with thickened endodermis cell walls exhibited strong birefringence. The occurrence of birefringent endodermis walls was most frequent in MPS and MSS sections. *Helichrysum lanceolatum* and *H. filicaule* were also found to exhibit strong birefringence in the tip sections. Endodermis walls with strong birefringence were observed in all species of *Cassinia* and *Ozothamnus*, and some species of *Ewartia, Helichrysum* and *Raoulia.*

Endodermis walls thickened: Prominent thickening of the endodermis cell walls was observed to be characteristic of some species. In the young stem of most species the endodermis cells were thin-walled, but gradually thickened as the stem aged. In the mature stem, however, only four species were observed to lack thickened endodermis walls consistently (*Haastia pulvinaris, H. sinclairii, Anaphalis subrigida,* and *A. trinervis*). Species observed to have thickened endodermis walls in the tip sections were *Raoulia australis, Helichrysum lanceolatum* and *Gnaphalium audax.*

The thickened walls in most species were pale in colour indicating the presence of suberin and/or lignin.

In species with thickened walls the radial, radial and outer tangential or all walls may become thickened. In endodermis cells with radial or all cell walls thickened, the thickened walls were of approximately even thickness in any one cell (Plate 7D). In cells with both radial and tangential endodermis wall thickening, the radial walls were often observed to taper, the greatest width being observed at the outer end of the radial wall. This was especially prominent in some *Raoulia* species (e.g. *R. australis, R. hookeri, R. monroi*) (Plate 7C).

In the mature primary stem of *Helichrysum parvifolium* and *H. coralloides* the endodermis was observed to be composed of a mix of cells with no walls or all cell walls thickened

present in the same section. All other species, however, were observed to have only one kind of thickening present in any one section.

Endodermis cells with all walls thickened were especially characteristic of *Ozothamnus* and *Cassinia*, being found in all the species examined. Thickened radial walls were observed in species of *Raoulia, Gnaphalium,* and *Anaphalis*. Thickened radial and outer endodermis cell walls were observed in *Anaphalis, Ewartia, Helichrysum,* and *Raoulia.*

Type of cortex: The cortex for each species was classified into one of three types based on the cell size and arrangement of the constituent cells.

Most species had a cortex of an approximately homogeneous appearance (e.g. Plate 8A, B). In these species the cortex cells were of approximately even size throughout the cortex or changed only slightly in size with no distinct boundary between cell sizes.
The second type of cortex appearance was observed in *Helichrysum parvifolium, H. dimorphum* and *Raoulia australis*. In these species the cortex was divided into two clearly demarcated layers with large cells in the peripheral layer, and small cells composing the inner layer (Plate 8C, D).

The final type of cortex arrangement was the reverse of the second type, with smaller cells in a clearly delimited outer layer, and larger cells in the inner layer (Plate 8E, F). This cortex arrangement was observed in both species of *Cassinia, Ewartia sinclairii, Raoulia tenuicaulis*, and some woody species of *Helichrysum*. The outer layer of small cells were frequently observed to contain a large number of chloroplasts.

Lignified cells in cortex: Thick-walled lignified cells (as indicated by pale blue staining and strong birefringence) developed in the cortex of a number of species. These cells were most prominent in the mature primary stem sections, although lignified cells were also present in the cortex of tip sections of two species (*Raoulia australis* and *Helichrysum filicaule*). In most species that developed lignified cortex cells the lignified cells were apparent in the MPS sections. In nine species, however (*Leucogenes leontopodium, Rachelia glaria, Raoulia petriensis, Anaphalis* species, and one specimen each of *R. cinerea* and *Ewartia catipes*), lignified cells developed later, being observed only in the

MSS sections. A number of species that retained the cortex at maturity also developed lignified cortex cells.

The lignified cells occurred in the cortex as a few isolated cells (e.g. *Helichrysum lanceolatum*, Plate 7A, B - adjacent to endodermis), small groups of cells scattered around or on one side of the stem (e.g. *Raoulia tenuicaulis*; Plate 9C), or the entire cortex may become lignified (e.g. *R. subsericea*; Plate 9A, B). Of the species that developed lignified cortex cells in the mature primary stem only four were outside *Raoulia*. These were *Leucogenes grandiceps*, *Helichrysum filicaule, H. bellidioides*, and *Ewartia sinclairii*.

Type and position of spaces in cortex: The cortex of all species had small intercellular spaces. These occurred at the corners of cells, at the point where three or more cells met. They varied in frequency from occurring only a few times in the cortex in some species to being present at nearly every cell corner in others. In addition to small intercellular spaces some species characteristically developed more prominent spaces in the cortex. In *Gnaphalium traversii* and *G. involucratum*, the small intercellular spaces were enlarged so that a prominent intercellular space was present at the corner of each cell (Plate 10A). Another form of cortex space that was observed was the development of spaces under the epidermis. In the most simple form these prominent spaces developed directly between the epidermis and the cortex cells. These spaces varied in size from small, only extending across two or three cells, to large (e.g. Plate 10B, C). Spaces were also observed to develop near the surface of the stem, but separated from the epidermis by two layers of cortex cells (e.g. *Ozothamnus leptophyllus*, Plate 10D). Both of these types of spacing were frequently, but not exclusively, observed to be located at the position at which the next leaf was detaching. In a number of species the prominent surface spaces were observed to continue into the lower portion of the sheath (e.g. *Raoulia grandiflora*, Plate 15E, *R.* sp. "L" Plate 14F).

Aerenchyma: The cortex of the mature stem in *Ewartia meredithiae* was found to be aerenchymatous, containing conspicuous and often large spaces between the cortex cells. (Plate 10E). The intercellular spaces appear to have developed lysogenically as indicated by the presence of short pieces of cell wall often observed protruding into the intercellular spaces (Plate 10F). The development of an aerenchymatous cortex was observed in the

MSS sections of both specimens of *E. meredithiae*, but was not observed in any other species.

Resin canals: Resin canals bordered by epithelial cells were found in the stem and sheathing leaf base of both *Haastia* species examined. The canals were associated with vascular tissue, being found either immediately adjacent to the endodermis in the stem (Plate 7E, F), or on the abaxial side of veins in the leaf sheaths. Resin canals appear to be the endodermal type of Solereder (1908) since the endodermis cells were included amongst the cells bordering the canals, on the side adjacent to the stele. Resin canals were lost due to development of the periderm in both mature specimens of *H. pulvinaris*.

Epidermis cell shape (Inc): The shape of epidermis cells in tip and MPS sections were classified as square, flat (radial dimensions < tangential dimensions), tall (radial > tangential), squashed, or any combination of the four categories. The epidermal cell shape varied, with no apparent pattern, both between specimens of the same species, and between different stages (i.e. tip versus MPS) of a single specimen. The only species that was found to have any consistency was *Raoulia glabra;* all young sections of this species were found to have a combination of flat and square epidermal cells.

Stomata in epidermis: Stomata were observed consistently in young sections of 16 species (See character 42 in Appendix 3). Stomata were also found in one sample of three other species; *Raoulia cinerea, Helichrysum bellidioides* and *Ewartia catipes*. Stomata in most species were presented approximately level with the surrounding epidermis (e.g. Plate 9C, D). However, prominently raised stomata were observed in *Cassinia* and *Ozothamnus* species (Plate 9E). Both raised and level stomata were found in one specimen each of *Cassinia longifolia* and *Ozothamnus obcordatus*.

Cuticle ridges and striations (Inc): Ridges in the cuticle were observed in tip and MPS sections of 25 species. In all specimens the ridges appeared to be caused by differences in the thickness of the cuticle layer.

The most frequently observed pattern of ridging (type A) was one in which the ridges were of unequal height and distribution (e.g. Plate 11A) . This contrasted with the even pattern of ridges in types B and C. In type B the cuticle appeared to be generally thick, but was

marked by distinct troughs. These troughs occurred at points on the cuticle corresponding to the junction of two epidermal cells (Plate 11B). Type C showed a similar pattern of troughs, but also had two or three distinct smaller ridges or points superimposed on the main ridge (Plate 11C).

In addition to cuticle ridges, some species were also observed to have striations in the cuticle. These occurred as distinctly darker, thin bands passing radially through the cuticle (Plate 11D) .

The occurrence of ridges and striations was inconsistent. Striations only occurred consistently in *Raoulia tenuicaulis* and *R.* sp. "M", but were also observed in some species of *Anaphalis, Ewartia, Leucogenes*, and *Ozothamnus*. Type A ridges occurred in some species of all but five genera (*Anaphalis, Haastia, Leucogenes, Pterygopappus, Pseudognaphalium*), although they were only found consistently in *Raoulia monroi, R. cinerea, R. glabra*, and *Ozothamnus obcordatus*. Type B ridges were observed consistently only in *O. rodwayi*, but were also observed in *Helichrysum coralloides, H. depressum, H. dimorphum*, and *R. subsericea*. Type C ridges were observed only in *Anaphalis keriensis, A. subridga,* and *C. aculeata.*

Biseriate hairs (Unc.): Two kinds of biseriate hairs were observed. The first type of hair normally contained four to six pairs of cells, and was characterised by prominent swelling of the terminal pair of cells (Plate 11F). The second type was usually formed by two rows of six to eight cells that gradually decreased in width, so that the hair tapered from the widest point adjacent to the epidermis, to the narrowest point at the tip of the hair (Plate 11E).

In transverse section only occasionally were the hairs oriented so as to provide a complete profile as seen in the photographs (Plate 11E, F). The presence of biseriate hairs was most frequently indicated by the occurrence of an infinity shaped (i.e. ∞) section through the hairs. It was therefore uncertain from transverse sections of most species, whether the specimens possessed one or both types of biseriate hair. Despite uncertainty as to the type hairs present in most species some trends were apparent.

Biseriate hairs were not observed in *Haastia pulvinaris, Raoulia haastii, R. hectorii* and *R.* sp. "M", but were observed in the tip sections of all other species. In the MPS sections, however, biseriate hairs were not observed in 30 of the 46 species in which they had been observed in the tip sections. In the MPS sections of *Raoulia,* for example, biseriate hairs were observed in only one of the two specimens of each of *R. cinerea, R. hookeri, R. subulata* Hook.f. and *R. glabra.*

In species where the type of biseriate hairs could be determined, both kinds of hairs were observed to occur together only in *Cassinia longifolia* and *C. aculeata.* Biseriate hairs with swollen terminal cells appeared to be the only type of biseriate hair in *Ozothamnus rodwayi, O. obcordatus, O. leptophyllus,* and *Helichrysum lanceolatum.*

Outer layer at maturity: The outermost layer in the mature stem was found to be the original epidermis, the endodermis, the cortex, and/or a periderm.

The original epidermis remained as the outer layer in a large number of species, but principally those which did not show a significant increase in the stem diameter. Thus, the epidermis exclusively formed the outer layer in all *Gnaphalium* and *Ewartia* species (excluding *E. sinclairii*), plus some species of *Raoulia* (e.g. *R. cinerea, R. grandiflora, R. monroi*), *Pseudognaphalium luteoalbum* and *Pterygopappus lawrencei.*

Cortex cells never occurred exclusively as an outer layer. Cortical cells occurred at the stem surface only as the result of breaks in the epidermis, or as remnant patches at the surface of species in which the periderm or endodermis principally formed the outer layer.

The endodermis was found to form part of the outer layer in only a few species, and the predominant part in only four species: *Raoulia subsericea, R. australis, R. glabra,* and *R. hookeri* (Plate 12A, B). In the last three species very weak periderm formation, or suberisation/lignification of the pericycle cells, was also noted. The endodermis was also noted as forming part of the outer layer in *Helichrysum bellidioides, R. tenuicaulis, Leucogenes grandiceps* and *H. lanceolatum.* In the last three species, however, the occurrence of the endodermis in the outer layer almost certainly represents a temporary stage following the loss of the cortex and epidermis, but prior to full periderm formation. The status of endodermis as the outer layer in *H. bellidioides* is less clear, since no

indications of periderm formation were noted, and one of the two specimens still retained the epidermis intact.

The periderm was noted as the major, or only, constituent of the outer layer of 26 species (Plate 12C, D, E, F). This included all species of *Ozothamnus*, *Cassinia*, and all woody species of *Helichrysum*. Periderm also occurred in the species of *Raoulia* which had large increases in stem diameter due to secondary growth (e.g. *R. eximia*, *R. haastii*). In many of these species, although the periderm was the major component of the outer layer, sometimes small areas of epidermis, cortex, and/or endodermis also were present (e.g. Plate 12C).

Periderm position: In species that were observed to develop a periderm, the periderm initially appears to develop in the outer cortex. As the stem aged further, however, the periderm generally became more deep seated. Thus, in most species examined, the periderm(if present) was located amongst the outer secondary phloem (e.g. *Helichrysum depressum,* Plate 12E; *Ozothamnus leptophyllus*, Plate 12C). In a few species the periderm remains largely superficial with the periderm retained in the outer cortex (e.g. *Haastia sinclairii*, Plate 12D; *Raoulia youngii*). *Helichrysum depressum* developed a distinct periderm in which successive layers were produced divided by weak parenchymatous cells. This results in a bark that contains several distinct layers (Plate 12E, F). The periderm in all species was thin, normally formed by no more than six to eight cells per layer. Cells immediately inside the periderm layer could not be distinguished from cortex or phloem cells, therefore no distinct phelloderm or phellogen could not be identified in most species.

Nodal anatomy: All species examined exhibited one of three types of nodal anatomy, all of which showed distinct leaf gaps. The three types of node found were unilacunar (with one trace from a single gap), trilacunar (with three (or more) leaf traces from three distinct gaps), and multilacunar (with more than three leaf traces from more than three gaps). The nodal type was consistent within a given species with two exceptions. *Ozothamnus leptophyllus* was found to have nodes that varied from unilacunar through to trilacunar. This was inconsistent even within a single specimen with one, two or three traces associated with an equal number of gaps (Plate 13A). The other exception, *Raoulia tenuicaulis*, was also inconsistent within a specimen, but only varied between two or three leaf traces and an equal number of gaps.

Trilacunar nodes were found in 34 of the 50 species (Plate 13B, E). This included all species of *Gnaphalium*, *Leucogenes*, *Rachelia*, *Haastia*, and *Cassinia*. Trilacunar nodes were also found in most species of *Raoulia* subg. *Raoulia* (excluding *R. australis*, *R. haastii*, and *R.* sp. "M", all unilacunar), and three species of *Ewartia* (the exception being *E. meredithiae*).
The trilacunar state was also found in three of the four species of *Anaphalis*. The exception, *A. rupestris*, was the only species found to have a multilacunar state, with five distinct leaf gaps and traces. (Plate 13C, D)

Unilacunar nodes were predominant in *Raoulia* subg. *Psychrophyton* (excluding *R. youngii, R. grandiflora*, and *R. hectorii*) and *Helichrysum* (excluding *H. bellidioides*, *H. filicaule*, and *H. lanceolatum*) (Plate 13F).

Arrangement of leaf traces (Inc): The arrangement of leaf traces in the tri- and multilacunar nodes was divided into two groups based upon the position of the laterals in comparison to the median trace. In the first group the laterals were observed to be ahead of the median trace, detaching from the vascular cylinder earlier than the median trace, and remaining in a more peripheral position throughout subsequent sections until in the leaf sheath. This was the most common of the two trace arrangements. In the second group the leaf traces all detached from the vascular cylinder at approximately the same time and remained approximately level in subsequent sections until in the leaf sheath.
This character was consistent in only *Cassinia longifolia, Ozothamnus obcordatus, Raoulia grandiflora,* and *Anaphalis rupestris.* In all other trilacunar species the arrangement of leaf traces varied both between specimens of the same species, and stages of the same specimen.

Number of veins in leaf sheath: In most species the number of veins in the leaf base was equal to the number of leaf gaps. In some species, however, the number of veins in the leaf base exceeded the number of leaf gaps. This resulted from the splitting of the main vein (e.g. *Helichrysum dimorphum*), the lateral veins (e.g. *Gnaphalium involucratum*, *Haastia pulvinaris*), or in some cases both the lateral and main veins (e.g. *Ozothamnus obcordatus* and *O. rodwayi*).

In most species that showed an increase in the number of veins, the increase occurred after the sheath base had fully detached from the stem (Plate 14C). In *Pseudognaphalium luteoalbum* and *Haastia sinclairii,* however, the lateral veins divided in the cortex prior to the sheathing base detaching from the stem (Plate 14A, B).

Distribution of sclerenchyma in leaf sheath: Sclerified cells (as indicated by the pale staining and strong birefringence) occurred in the leaf sheath (excluding the veins) of 27 species. The sclerified cells occurred in the adaxial epidermis and/or the mesophyll with different distributions characteristic of certain species.

The most frequently observed sheath cells to become sclerifed were the mesophyll cells. In some species a few isolated sclerified cells occurred in the mesophyll (e.g. *Cassinia longifolia, Haastia pulvinaris, Raoulia cinerea*). By comparison a large number of sclerified cells arranged in groups were observed in the mesophyll of *Raoulia glabra, R. subsericea,* and *R. monroi*. The entire mesophyll consistently became lignified in *R. mammillaris, R. haastii, R. australis* and *Ozothamnus leptophyllus* (Plate 14D).

The distribution of sclerified cells in the leaf sheath of *Gnaphalium nitidulum*, *Raoulia hectorii*, and *R. subulata* was much more discrete, occurring as a single cell layer at the top of the mesophyll, immediately adjacent to the adaxial epidermis (Plate 14E). In *G. nitidulum* the sclereids spread across the entire leaf width, but in *R. hectorii* they were restricted to the area between the lateral veins. *Raoulia subulata* was intermediate between *R. hectorii* and *G. nitidulum*.

Ewartia meredithiae and *Raoulia* sp. "L" were the only species in which the entire adaxial epidermis was observed to become sclerified (Plate 14F). In both specimens of *E. meredithiae* and one specimen of *R.* sp. "L" the lower mesophyll layer also became lignified. A few scattered sclerified cells occurred in the adaxial epidermis of *R. hookeri, R. mammillaris, R. australis* and *Ozothamnus leptophyllus* (only in one specimen of each).

Of the 27 species in which sclerified cells were observed in the leaf sheath, only eight species were found to be inconsistent for the occurrence of sclereids. These species were *Leucogenes grandiceps*, *Cassinia longifolia, Haastia pulvinaris, Helichrysum coralloides*

H. filicaule, Ozothamnus obcordatus, Pterygopappus lawrencei, and *Raoulia cinerea.* In all of these species the sclerified cells occurred as a few scattered cells in the mesophyll, except *L. grandiceps* in which they were restricted to the adaxial mesophyll.

Number of bundle sheath layers: In all species the veins of the leaf sheath were surrounded by a bundle sheath. The bundle sheath appeared to be derived from the endodermis with endodermis cells surrounding the leaf traces as they separated from the vascular cylinder. In all species, except those of *Anaphalis,* the bundle sheath appeared to consist of single layer of parenchymatous cells. The four *Anaphalis* species were observed to have a double layer of cells surrounding the sheath veins. This coincided with the occurrence of two layers of endodermis cells in the tip sections, one of which was lost in the older sections (MPS and MSS sections) (Plate 13D).

Sclerified bundle sheath cells: Sclerenchymatous cells occurred in the bundle sheaths of seven species (*Ozothamnus leptophyllus*, *Helichrysum filicaule*, *H. lanceolatum, E. planchonii, E. meredithiae*, *Raoulia subulata*, and *R. hectorii*). In all species, except *H. filicaule* and *E. planchonii*, the presence of sclerenchymatous bundle sheath cells was consistent.

Sclerenchymatous cells in *Ozothamnus leptophyllus* and *Helichrysum filicaule* occurred as a few thick walled cells at points around the bundle sheath. By comparison, in *Helichrysum lanceolatum* most of the bundle sheath cells were sclerified, with only a few parenchymatous cells included in the sheath (Plate 15B). In the four other species observed to have sclerenchymatous bundle sheath cells (*Ewartia meredithiae, E. planchonii*, *Raoulia subulata*, and *R. hectorii*), only the cells in the adaxial part of the bundle sheath became sclerified.

Sclerenchyma bundle caps: Sclerenchyma caps occurred in the mid vein of 11 species as abaxial or adaxial caps, or as a complete cylinder. Abaxial sclerenchyma caps were observed in all the woody species of *Helichrysum,* except *H. depressum* and *H. dimorphum*, and in one specimen of *Raoulia bryoides*. The cells forming the caps of *H. intermedium, H. parvifolium*, and *H. coralloides* were extremely thick walled, with only a few small cell lumen present (Plate 15A). In *H. lanceolatum*, while the cell walls were prominently thickened, a large lumen remained visible (Plate 15B).

Sclerenchyma caps on the adaxial side were observed in *Raoulia subulata*, *R.* sp. "L", *R. grandiflora*, and *Ewartia planchonii*. In *R. grandiflora* both the lateral and main veins had distinct sclerenchymatous caps (Plate 15E). Both adaxial and abaxial caps were observed in the main vein of *Leucogenes leontopodium* (Plate 15C).

Ewartia meredithiae was unique amongst the species examined, in that the sclerenchyma cells formed a complete cylinder around the main vein, immediately inside the bundle sheath (Plate 15D).

Crystals and starch grains (Inc): Using polarised light, crystals were located in the pith and cortex of 21 species. The crystals were always extremely small (<< 10 μm) and normally solitary. Irregular crystalline bodies in the cells of a few species, however, possibly indicated the presence of a group of thin rod shaped crystals. (This could not be confirmed.) Crystalline bodies with an elliptical shape and a rough surface were located in one specimen each of *Gnaphalium audax* and *G. mackayi*. These crystals appear to match the descriptions of silica bodies (Plate 16A).

Three other types of crystals were also found. These were categorised into spherical, rhomboidal, and rod-shaped crystals. The rhomboidal crystals varied in outline shape from square to rectangular to truly rhomboidal. All, however, were observed to have only four sides (as viewed in outline) (Plate 16B). A rhomboidal crystal located in *Helichrysum intermedium* under SEM (Plate 16C) was analysed using the Oxford Energy Dispersion unit. The resulting spectra showed peaks corresponding to magnesium and oxygen (Figure 2.1). Rhomboidal crystals were observed in 10 taxa including some species in each of *Helichrysum*, *Gnaphalium*, *Cassinia, Anaphalis*, and one specimen of *Ewartia sinclairii*.

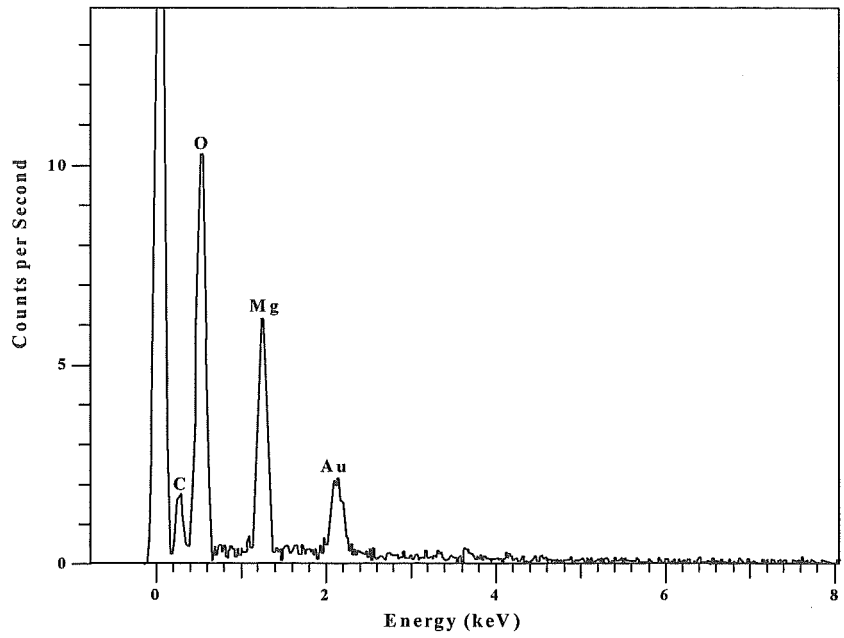


Figure 2.1: Spectra produced from energy dispersion analysis of a crystal located in *Helichrysum intermedium* showing peaks corresponding to Oxygen and Magnesium.

Spherical crystals (Plate 16E) were observed in 10 taxa including *Rachelia glaria*, both species of *Haastia*, and some taxa of *Anaphalis*, *Helichrysum*, *Ozothamnus*, and *Pseudognaphalium*. Spherical crystals were readily distinguished from starch grains by the absence of the distinctive Maltese cross associated with starch grains.

Rod shaped crystals (Plate 16D) were less common than spherical and rhomboidal crystals, being observed in only five species all belonging to different genera (see Appendix 3). The crystals were all short (<10 μm in length) and extremely thin (width estimated at < 1 μm).

The presence of crystals within a species was inconsistent. Crystals of the same kind were observed only in both specimens of *Cassinia aculeata* and *Helichrysum coralloides*. In all other instances where crystals were observed only one specimen contained crystals, or crystals of different kinds were observed in each specimen.

Starch grains (Plate 16F) were observed in the cortex and leaf sheath of five taxa; *Cassinia longifolia*, *Haastia pulvinaris*, *Helichrysum depressum*, *H. dimorphum*, and *Ozothamnus*

obcordatus. The starch grains varied in size, but were all spherical in outline and less than 10 μm in diameter.

Dimensions of the mature stems: The stems were found to range in maximum radius from 3 mm (e.g. *Raoulia* sp. "M", *Pterygopappus lawrencei*) to over 30 mm (e.g. *R. eximia, Ozothamnus leptophyllus*). Most species however, ranged between 6 and 12 mm in radius. (All measurements were taken from the centre of the pith to the outside of the stem along the maximum radius. The stem radius can therefore not be doubled to give a stem diameter.) The proportion of xylem in the stem had a significant positive correlation with the stem diameter ($r = 0.620$, $p < 0.001$), indicating that in many species with large stems a high proportion of increase in stem radius was due to xylem production (Figure 2.2:A). However, in some species with a large stem radius (e.g. *Anaphalis* species and some *Gnaphalium* species), the pith and cortex were the major components in the stem radius. The proportion of stem occupied by the pith varied from less than 10% (e.g. *O. leptophyllus, R. eximia, Leucogenes leontopodium*) to over 50% (e.g. *Helichrysum filicaule, A. trinervis, G. involucratum*). In comparison the xylem varied from 4% of the stem radius (e.g. *Ewartia planchonii*) to over 70% (e.g. *H. intermedium, R. haastii*). The proportion of the mature stem occupied by xylem exhibited a significant negative relationship to the proportion occupied by pith ($r = -0.664$, $p < 0.001$) (Figure 2.2:B).

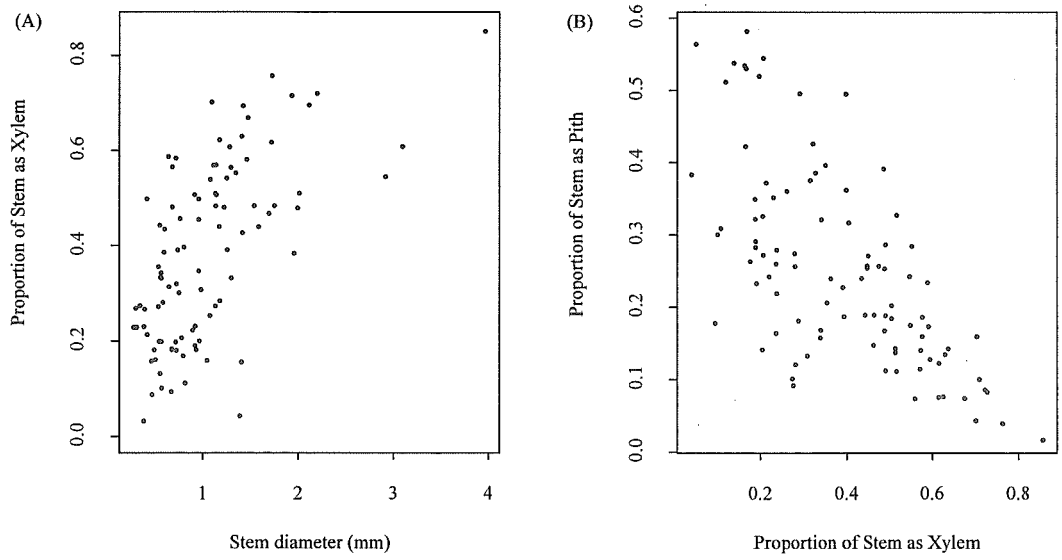


Figure 2.2: Scatter graphs showing (A) the positive relationship between stem diameter (mm) and the proportion of the stem radius occupied by the xylem, and (B) the negative relationship between the proportion of the stem radius occupied by the xylem and the pith.

Abbreviations of species names in Cladistic and Numerical Results

In all dendrograms (whether cladistic or phenetic) species names are abbreviated to six letters by using the first three letters of the genus and species names. The only exception is *Raoulia subulata*, which is abbreviated to Raosuu to avoid confusion with *R. subsericea* (Raosub).

In order to save space in the PCoA plots, the species are indicated by the first letter of the genus name and three letters of the species name, with two exceptions. *Raoulia subulata* is abbreviated to Rsuu, again to avoid confusion with *R. subsericea* (Rsub), and *Rachelia glaria* is abbreviated to Rgar to avoid confusion with *Raoulia glabra* (Rgla).

2.3.2 Cladistic analyses

The five searches with the maxtree limit set to 3 000 all reached the maxtree limit at least once, and returned 3 000 equally parsimonious trees. Searches 2, 3 and 5 each returned trees of length 352, whilst searches 1 and 4 produced trees that were two steps longer (Table 2.2). The symmetrical distances between the strict consensus trees generated by each search (Table 2.2) indicate that each of the five searches located a different island. This was confirmed by loading the saved tree files (which contained all 3 000 trees for each search) one against the other using the filter in Paup to remove any trees in common. There were no trees shared between any of the 3 000 trees from each search, indicating that each search probably identified a unique local optimum, or island. These will be referred to as Islands 1 to 5. The strict consensus trees for the five islands are presented in Figure 2.3 to Figure 2.7.

Search	1	2	3	4	5	6
1	-					
2	24	-				
3	35	17	-			
4	19	13	18	-		
5	32	10	21	19	-	
6	32	10	21	19	0	-
Length	354	352	352	354	352	352

Table 2.2: Symmetrical distances between the strict consensus trees found by six randomly generated heuristic searches in Paup, and the length of the equally parsimonious trees identified by each search.

The single search with maxtree set to 10 000 (Run 6), also reached the maxtree limit at least once. Comparing the symmetrical differences between the strict consensus trees (Table 2.2) indicates that this search identified the local optima corresponding to Island 5. This was confirmed by loading the two tree files against one another. Of the 3 000 trees identified by Island 5 only 94 were not included in the 10 000 trees found by Run 6.

Comparison of the 5 Islands

The strict consensus trees from the five islands showed marked structural differences, especially when Islands 1 and 4 are compared to Islands 2, 3, and 5. The major structural difference in Island 1 is the position of the clade formed by *Haastia* and woody species of *Helichrysum* (excluding *H. lanceolatum*) and the clade formed by *Raoulia bryoides, R. haastii,* and *R. mammillaris*. In Islands 2 to 5 these two clades occur on a polychotomy towards the base of the tree. In Island 1 these two clades occur further up the tree, in a large group containing other species of *Raoulia*, both species of *Leucogenes*, and *Ewartia catipes*.

The strict consensus tree from Island 4 differs from the other consensus trees in that it is less resolved. It contains two main polychotomies, with only four smaller clades present in the larger of the these. Most other structural differences between the five Islands result from taxa being placed in different arrangements within the same polychotomy. For example the difference between Islands 2 and 5 is the arrangement of the taxa in the terminal polychotomy. In Island 5, but not Island 2, *Raoulia tenuicaulis*, *Gnaphalium audax* and *Pseudognaphalium luteoalbum* form a clade with *R. monroi, R. glabra* and *Helichrysum filicaule*. In the same terminal polychotomy *H. bellidioides, R. petriensis*, and *R. youngii* also form a clade in Island 5, but not Island 2.

Despite the differences between the Islands some consistent features are present, including four clades which occur in all Islands. These are:

(1) The large clade formed by all species except those belonging to *Cassinia* and *Ozothamnus*. Thus all the New Zealand taxa, except *O. leptophyllus*, form a clade with the Australian species of *Ewartia* and *Pterygopappus*.

(2) The clade formed by the four *Anaphalis* species occurs in all 5 Islands. The internal structure of this clade is also consistent in all Islands.

(3) *Haastia pulvinaris* and *H. sinclairii* form a clade in the consensus trees of each Island.

(4) *Raoulia mammillaris* and *R. bryoides* form a clade to which *R. haastii* is a sister taxon in all Islands.

Other groupings occur in three or four of the five Islands. Three of these are:

(1) The grouping of *Ewartia sinclairii* and *Helichrysum lanceolatum* as a clade, or as leaves on the same polychotomy, typically towards the base of the tree.

(2) The placement of *Leucogenes leontopodium*, *Raoulia subulata* and *R. hectorii* in a polychotomy which is basal to the large polychotomy containing *Anaphalis, Gnaphalium* and some *Raoulia* species.

(3) the clade group formed by *Raoulia monroi* and *R. glabra*, to which *Helichrysum filicaule* is a sister taxon in three of the five strict Islands.

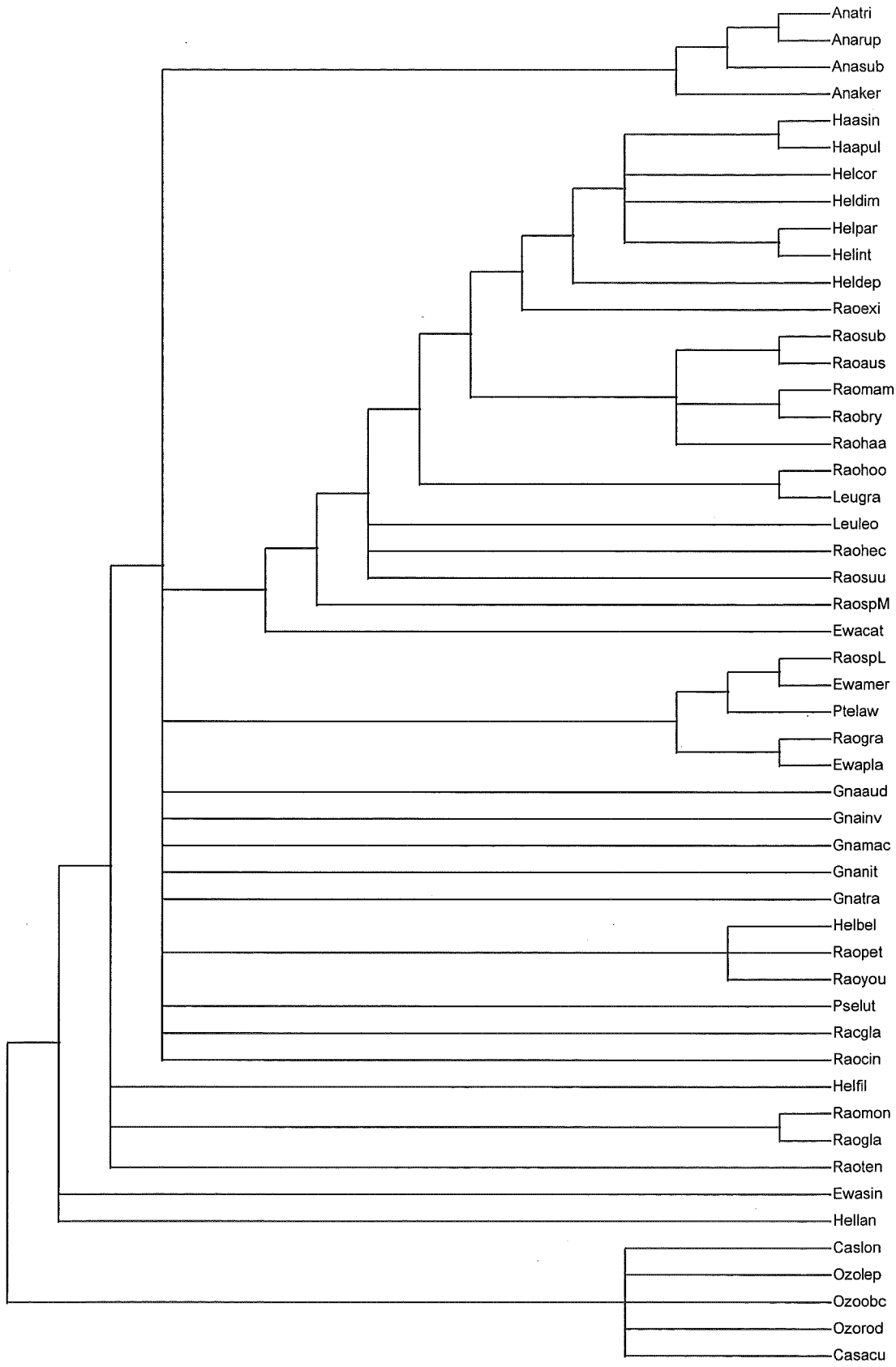


Figure 2.3: Strict consensus tree of 3 000 equally parsimonious trees found on Island 1.

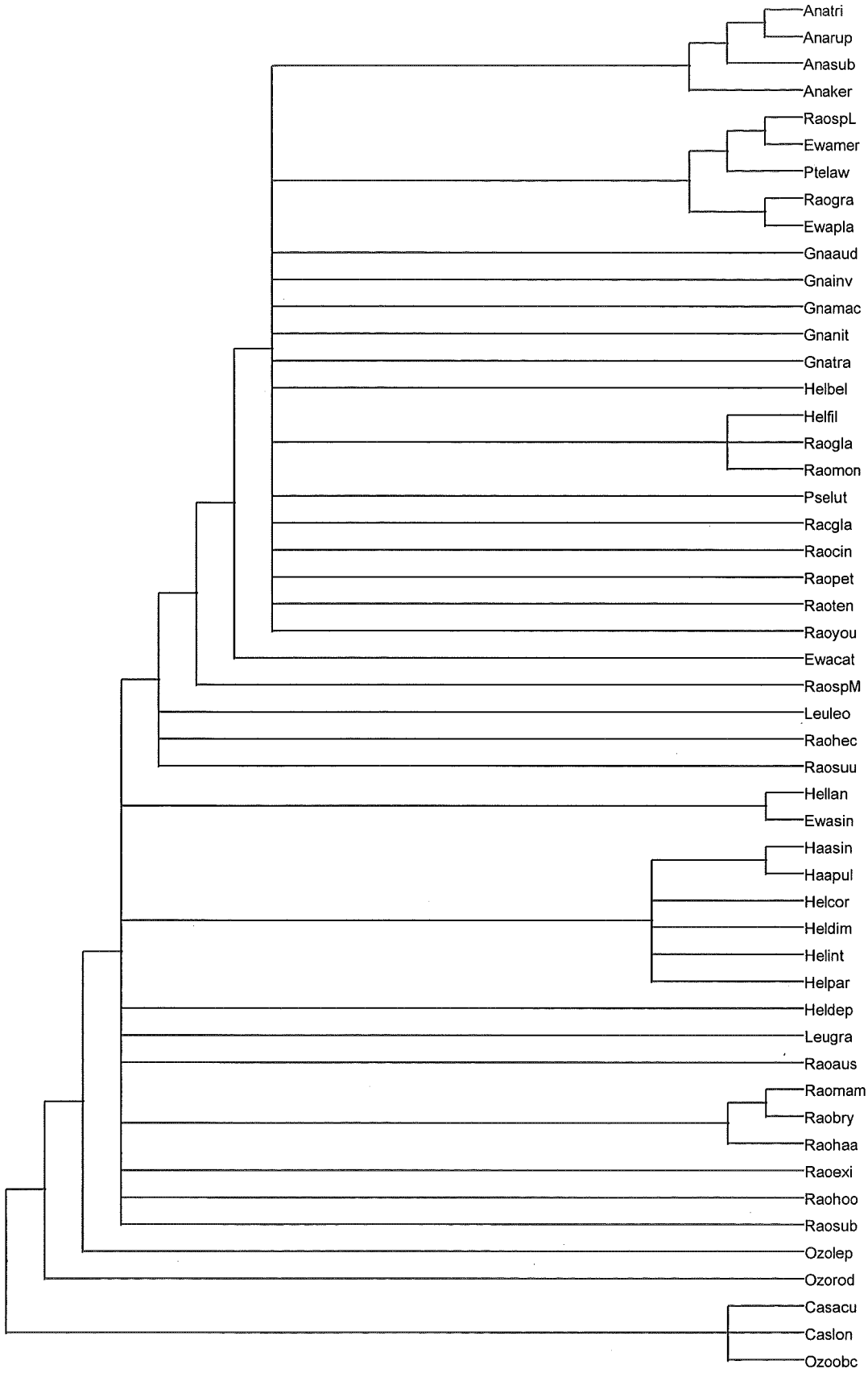


Figure 2.4: Strict consensus tree of 3 000 equally parsimonious trees found on Island 2.

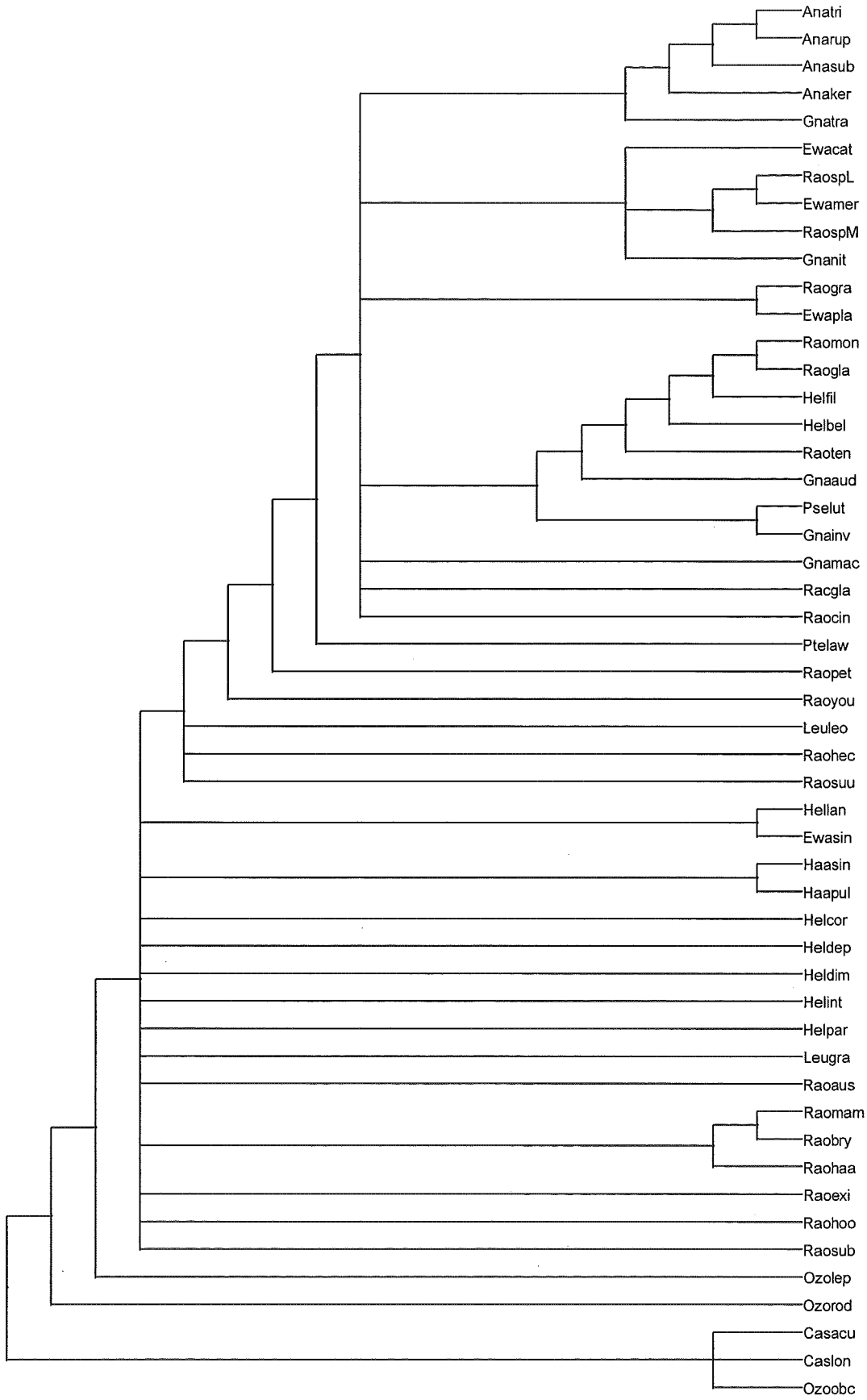


Figure 2.5: Strict consensus tree of 3 000 equally parsimonious trees found on Island 3.

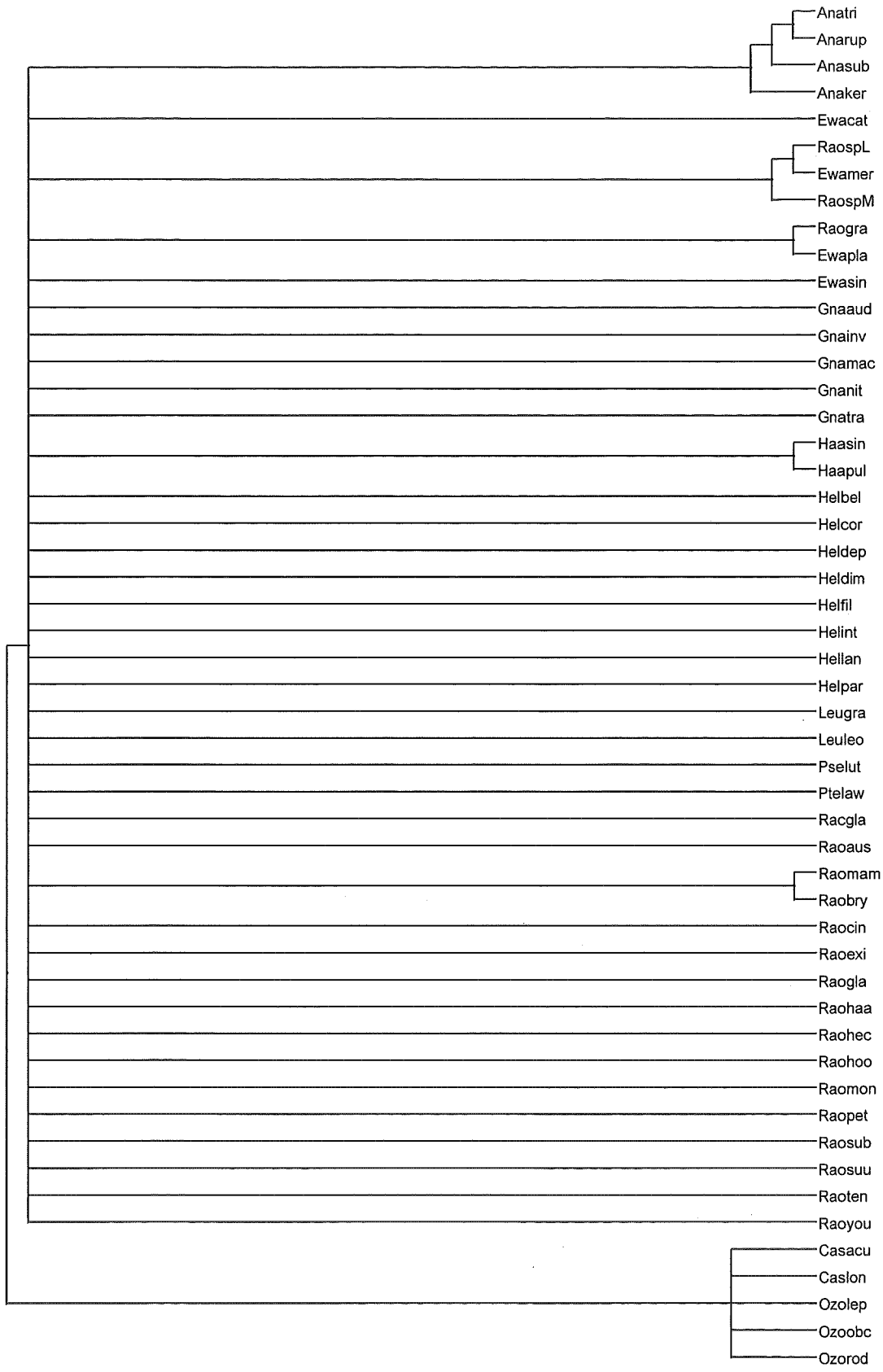


Figure 2.6: Strict consensus tree of 3 000 equally parsimonious trees found on Island 4.

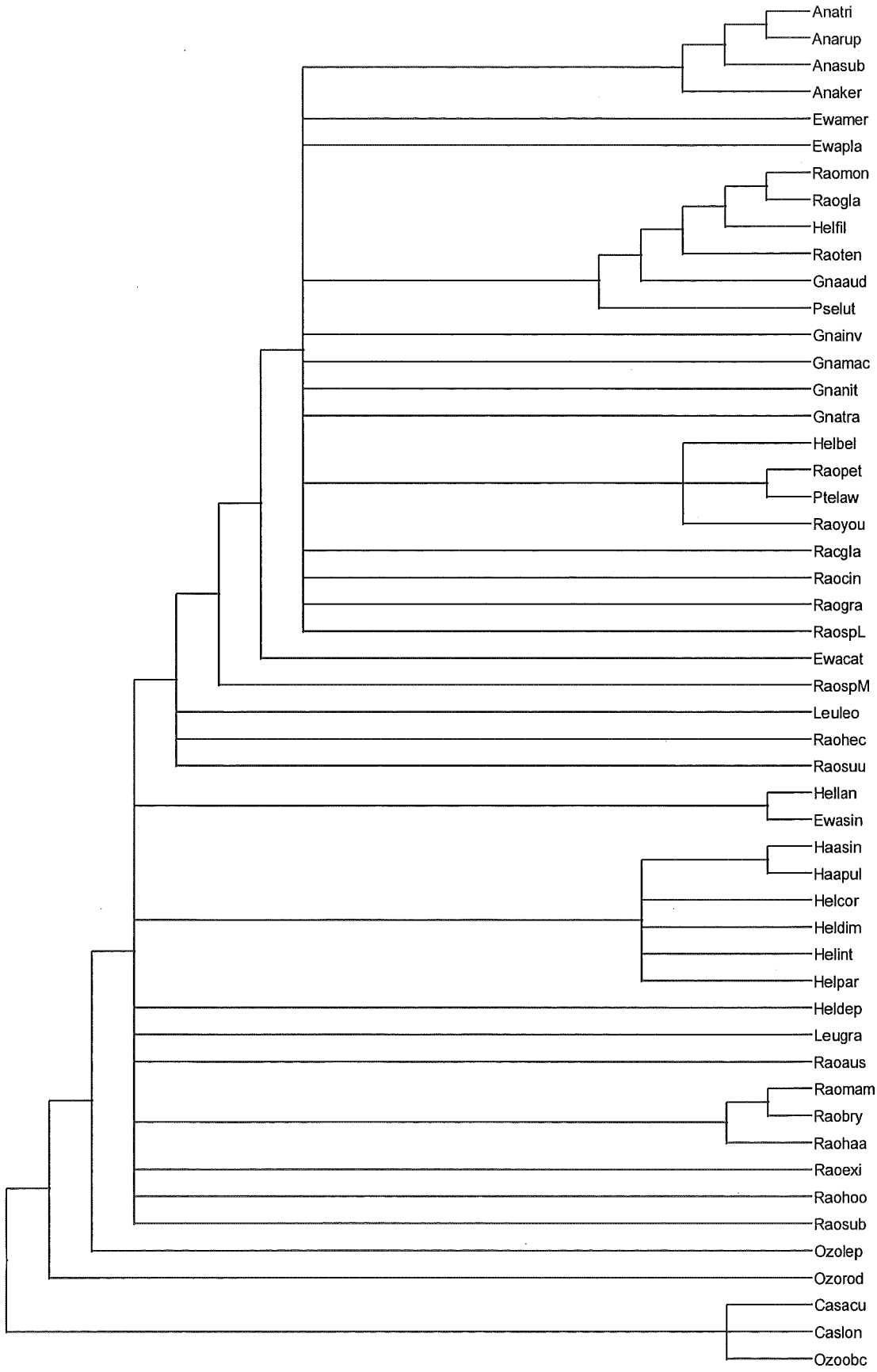


Figure 2.7: Strict consensus tree of 3 000 equally parsimonious trees found on Island 5.

Consensus tree from Run 6

The strict consensus tree from Run 6 (i.e. maxtree limit set to 10 000) was chosen to illustrate the distribution of character state changes (Figure 2.8). These are indicated on the tree for characters which had less than 10 steps. Characters which occurred only as parallelisms in the terminal branches are not shown since they do not contribute to the structure of the tree.

Of the seven apomorphies which occur on the tree without reversal only three occur as synapomorphies, and therefore may be considered to characterise monophyletic groups. The occurrence of flat stomata (Character 43) separates *Cassinia* and *Ozothamnus* from all the other taxa. The presence of resin canals (Character 31) characterises the two species of *Haastia*, while the occurrence of two bundle sheath layers (Character 60) is one of the characters which defines the clade formed by *Anaphalis*. All other clades on the tree are defined by characters which show reversals and/or parallelisms.

Comparing the distribution of the genera on the tree, only *Anaphalis* and *Haastia* form monophyletic groups.

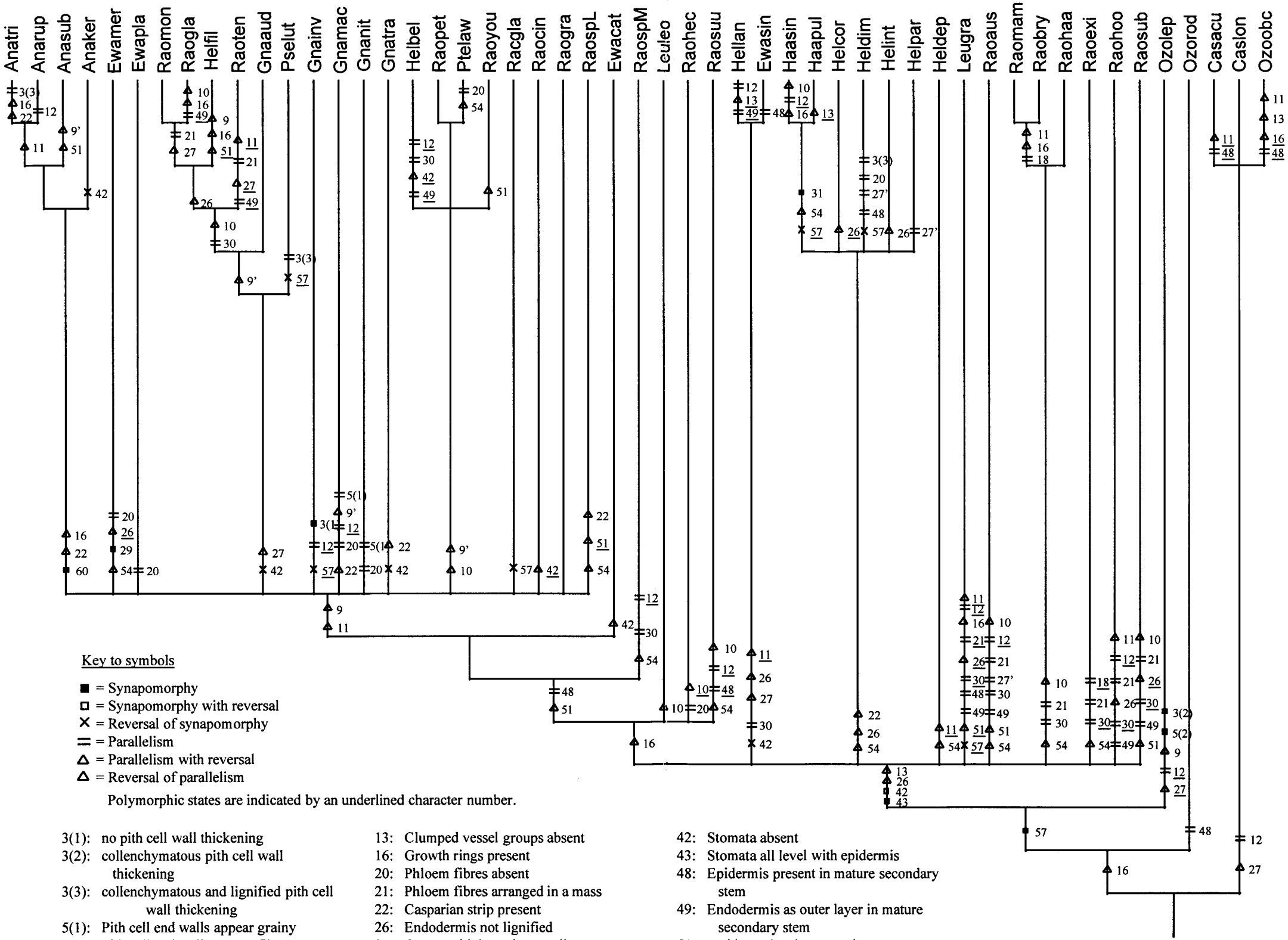
Four woody species of *Helichrysum* (*H. parvifolium, H. intermedium, H. coralloides, H. dimorphum*) form a polychotomy that includes *Haastia* and is defined by the presence of a casparian strip, lignification of the endodermis, and a unilacunar nodal state (a feature which reverses in *Haastia*). The other two woody species of *Helichrysum* both belong to the same large polychotomy as the small clade above. *Helichrysum depressum* occurs as an individual branch, while *H. lanceolatum* forms a small group with *Ewartia sinclairii*. The two herbaceous species of *Helichrysum* do not form a monophyletic group, although both occur in the same large terminal polychotomy which is defined by an unlignifed middle lamella in the pith (character 9) and the absence of tangential vessel groupings (character 11). This large polychotomy also includes all species of *Gnaphalium, Pseudognaphalium, Rachelia*, *Pterygopappus*, and seven species of *Raoulia.*

The five species of *Gnaphalium* do not form a monophyletic group, with four of the five species occurring as individual terminal branches. The fifth species, *G. audax*, occurs as a sister taxon to the clade formed by *Raoulia tenuicaulis*, *R. glabra*, and *Helichrysum filicaule*.

Also included in the large terminal polychotomy are two species of *Ewartia*. *Ewartia meredithiae* and *E. planchonii* both occur as individual branches in this polychotomy. As mentioned above, *E. sinclairii* forms a group with *H. lanceolatum* lower in the tree. The fourth species of *Ewartia*, *E. catipes*, occurs as the sister taxon to the large terminal polychotomy. The clade formed by the addition of *E. catipes* to the terminal polychotomy is only supported by the type of cortex spacing (character 28). The state changes in this character are not shown since this character has 16 character states changes on the tree.

Raoulia does not form a monophyletic group. In *Raoulia* subg. *Raoulia* only *R. monroi, R. glabra* and *R. tenuicaulis* form a clade, in association with *Helichrysum filicaule*. This clade is characterised by lignified primary xylem (character 10) and the presence of lignified cortex cells (character 30). Of the six remaining species, one (*R. cinerea*) occurs in the same polychotomy as a single branch, while *R.* sp. "M" occurs as a sister taxon to this large polychotomy. Three of the remaining species occur as individual branches in a polychotomy near the base of the tree, whilst *Raoulia haastii* occurs as a sister taxon to two species of *Raoulia* subg. *Psychrophyton* (*R. bryoides* and *R. mammillaris*). This grouping of *R. mammillaris* and *R. bryoides* represents the only monophyletic grouping of *Raoulia* subg. *Psychrophyton* in the tree. *Raoulia eximia* occurs as an individual branch in the large polychotomy near the base of the tree. *Raoulia hectorii* and *R. subulata* occur in a small polychotomy with *Leucogenes leontopodium*, while *R.* sp. "L" and *R. grandiflora* occur as individual branches in the large terminal polychotomy. The remaining species of *Raoulia* subg. *Psychrophyton*, *R. youngii*, occurs in a clade with *Helichrysum bellidioides, R. petriensis* and *Pterygopappus*. This clade is defined by the lignification of the middle lamella (character 9') and primary xylem (10).

The species of *Cassinia* and *Ozothamnus* occur at the base of the tree. *Cassinia aculeata* and *Cassinia longifolia* form a polychotomy with *O. obcordatus*, while *O. rodwayi* and *O. leptophyllus* occur as sequential sister taxa to all the other species in this study.



3(1): no pith cell wall thickening
3(2): collenchymatous pith cell wall thickening
3(3): collenchymatous and lignified pith cell wall thickening
5(1): Pith cell end walls appear grainy
5(2): Pith cell end walls appear fibrous
9: Pith middle lamella dark (unlignified)
9': Pith middle lamella pale (lignified)
10: Primary xylem cell lignified
11: Tangential vessel groups absent
12: Radial vessel groups present
13: Clumped vessel groups absent
16: Growth rings present
20: Phloem fibres absent
21: Phloem fibres arranged in a mass
22: Casparian strip present
26: Endodermis not lignified
27: Cortex with large inner cells
27': Cortex with large outer cells
29: Cortex aerenchymatous
30: Lignified cells present in cortex
31: Resin canals present in cortex and leaf sheath
42: Stomata absent
43: Stomata all level with epidermis
48: Epidermis present in mature secondary stem
49: Endodermis as outer layer in mature secondary stem
51: Periderm development absent
54: Nodes unilacunar
57: Number of veins in leaf sheath equal to number of leaf traces
60: Two bundle sheath layers

Figure 2.8: The strict consensus tree of 10 000 trees of length 352 identified by Run 6, showing character state changes.

2.3.3 Numerical analyses

The Cophenetic correlation coefficient for the average linkage and single linkage trees were 0.6547 and 0.493 respectively. This indicates that the average linkage tree provides a better representation of the original similarity data matrix.

Average Linkage Dendrogram

The average linkage dendrogram (Figure 2.9) can be split into five main clusters, A to E.

Cluster A contains both species of *Haastia* joining at a relatively low level similarity (0.722), before linking more distantly with *Helichrysum dimorphum* at 0.606. This cluster is the last to join the rest of the tree formed by clusters B to E.

Cluster B includes all species of *Cassinia* and *Ozothamnus*, with the Australian taxa clustering sequentially, followed more distantly by *O. leptophyllus* (0.645).

Cluster C is composed of three smaller clusters (1 to 3). Cluster 1 contains *Helichrysum filicaule, H. lanceolatum*, and *Ewartia sinclairii*, all of which join at a relatively low level of similarity (0.711 and 0.669). Clusters 2 and 3 contain most of *Raoulia* subg. *Raoulia* plus *Leucogenes grandiceps* and *Helichrysum bellidioides*. Clusters 2 and 3 link together at 0.665, before linking with Cluster 1 at 0.629.

Cluster D is also composed of three smaller clusters (4 to 6) which join at 0.64. Cluster 4 is formed by a close pairing of *Raoulia bryoides* and *R. mammillaris* (which link at 0.895), which then cluster more remotely with *R. eximia* (0.744), and *R. haastii* (0.734). Cluster 5 contains all the woody species of *Helichrysum*, except *H. lanceolatum* and *H. dimorphum*, with the closest pairing being between *Helichrysum intermedium* and *H. parvifolium* (0.835).

Cluster E is formed by Clusters 8 and 9 linking with Cluster 7 at 0.645. Cluster 7 contains all species of *Anaphalis*, while Clusters 8 and 9 represent all species of *Gnaphalium,* plus *Pterygopappus lawrencei, Rachelia glaria*, two species of *Ewartia*, and the remaining *Raoulia* species. Cluster E is joined distantly by *Ewartia meredithiae*.

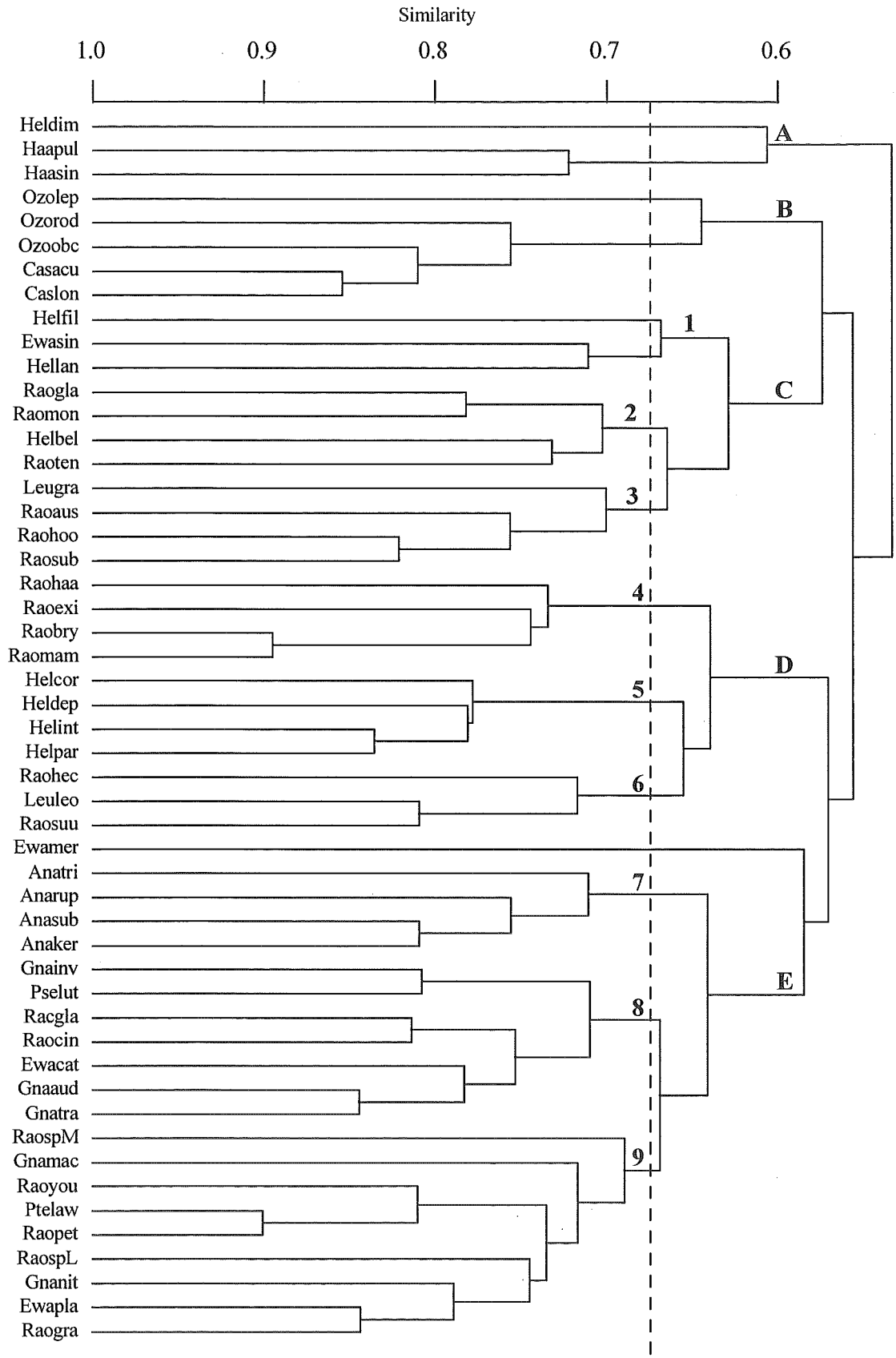


Figure 2.9: Average linkage dendrogram based on stem anatomy data using Gower's General Coefficient Similarity.

The Cophenetic correlation coefficient for the average linkage tree drops sharply with the addition of *Raoulia youngii* to the cluster of *Pterygopappus* and *R. petriensis* at 0.8102 (Figure 2.10). The correlation coefficient then stabilises with correlations between 0.712 to 0.794 for the merges occurring between 0.8098 and 0.6899. The last peak before the correlation coefficient begins to drop again occurs at 0.6695 (indicated by the broken line). This corresponds to the addition of *R.* sp. "M" to Cluster 9 (Figure 2.9). This peak represents the point on the dendrogram at which the correlation between the similarity matrix and dendrogram is still relatively high, and at which all but three taxa have been added to a cluster. On this basis the clusters formed up to this level were depicted on the PCoA plots. Following this peak the correlation between the dendrogram and the similarity matrix gradually drops, except for a small peak which corresponds to the clustering of groups D and E.

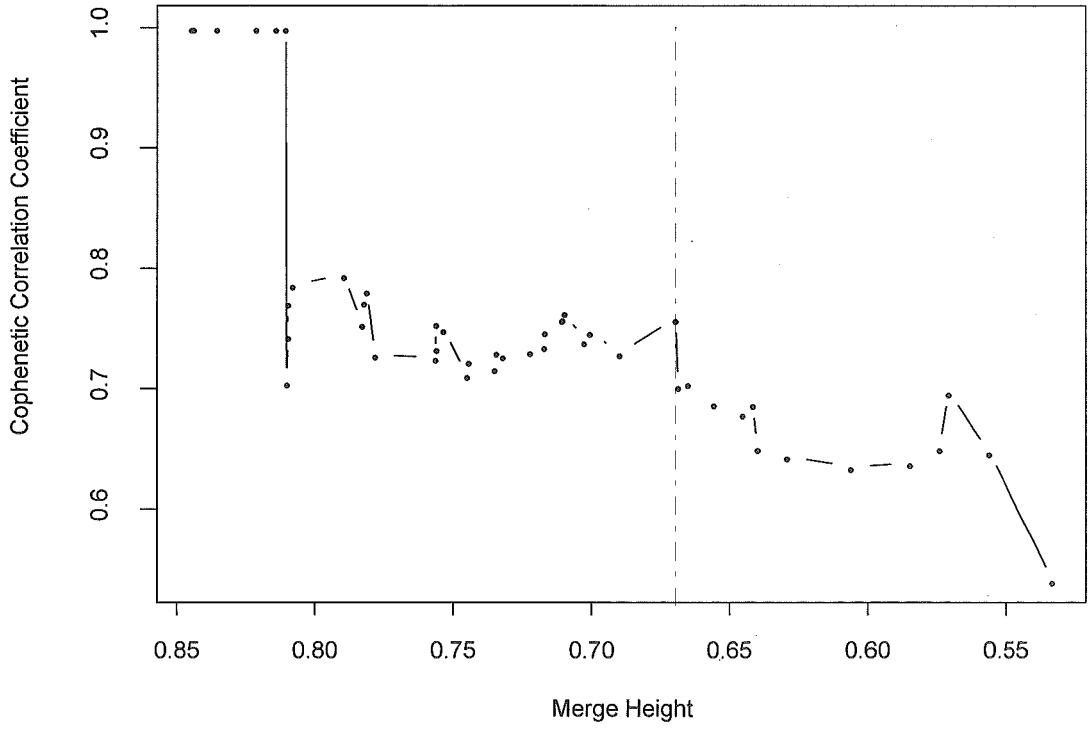


Figure 2.10: Plot showing the change in the Cophenetic correlation coefficient as taxa are clustered in the average linkage dendrogram.

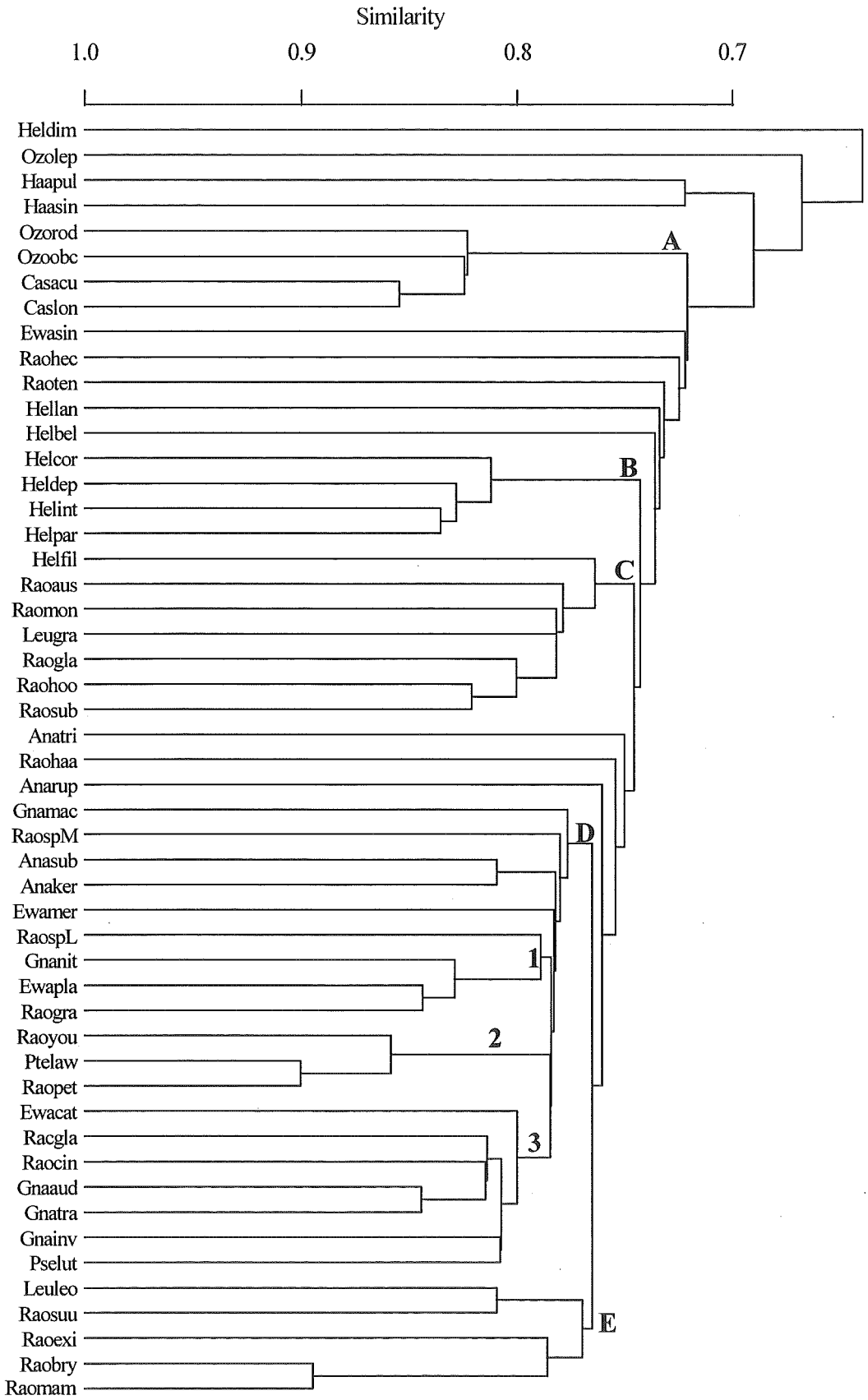


Figure 2.11: Single linkage dendrogram based on stem anatomy data using Gower's General Coefficient of Similarity.

Single Linkage Dendrogram

The single linkage dendrogram can be split into a number of isolated taxa plus five main groups (A to E) of varying sizes (Figure 2.11).

Cluster A is formed by the Tasmanian species of *Ozothamnus* and both species of *Cassinia,* all of which cluster by 0.823. Cluster B includes all the woody species of *Helichrysum*, except *H. lanceolatum* and *H. dimorphum*, with all four species in the cluster linking in a narrow range of similarity (0.8354 to 0.8122).

Cluster C contains most of *Raoulia* subg. *Raoulia*, as well as *Leucogenes grandiceps* and *Helichrysum filicaule*. Within this cluster *Raoulia subsericea*, *R. hookeri* and *R. glabra* link most closely (0.821 and 0.801). These three species are then linked to *R. monroi* and *L. grandiceps* at 0.7822, before clustering slightly more distantly to *R. australis* (0.782), and *H. filicaule* (0.764).

Cluster D is formed by three smaller clusters (Clusters 1 to 3), to which *Ewartia meredithiae*, *Anaphalis subrigida, A. keriensis, Raoulia* sp. "M", and *Gnaphalium mackayi* link more distantly. Cluster 1 is formed by *Raoulia grandiflora* and *E. planchonii* (0.844), joining sequentially with *G. nitidulum* (0.829) and *R.* sp. "L" (0.783). Cluster 2 contains only three species, *R. petriensis*, *Pterygopappus lawrencei* and *R. youngii*. The cluster between *R. petriensis* and *P. lawrencei* has the highest level of similarity on the tree (0.901). Cluster 3 contains *Gnaphalium audax, G. traversii* and *G. involucratum*, plus *R. cinerea*, *Rachelia glaria, Pseudognaphalium luteoalbum,* and *E. catipes*. All linkages in Cluster 3 occur between 0.845 and 0.800.

Cluster E is formed by two smaller clusters. *Leucogenes leontopodium* and *Raoulia subulata* cluster together at 0.8097, before joining at 0.77 with the cluster of *R. mammillaris*, *R. bryoides* and *R. eximia.*

A number of isolated taxa are present in the single linkage tree. The most isolated taxon is *Helichrysum dimorphum* which is the last species to be clustered, joining the other taxa at a low level of similarity (0.640). *Ozothamnus leptophyllus* is also isolated, linking to the other taxa immediately prior to *H. dimorphum* at 0.668.

Haastia pulvinaris and *H. sinclairii* cluster together at 0.722, but are otherwise isolated in the tree.

Between the union of Cluster A to B five reasonably isolated taxa (*Ewartia sinclairii, Raoulia hectorii, R. tenuicaulis, Helichrysum lanceolatum* and *H. bellidioides*) link sequentially at similarity levels of 0.734 to 0.722. A similar chain of slightly isolated taxa occurs prior to the linkage of Cluster C to Clusters D and E, in this case the isolated taxa are *Anaphalis trinervis*, *A. rupestris*, and *R. haastii*.

Comparison of Average and Single Linkage Dendrograms

On preliminary examination the overall structure of the two dendrograms appears to differ markedly, mainly due to the large number of taxa in isolated positions and the chaining in the single linkage tree. But, when the two dendrograms are examined more thoroughly a number of consistent clusters become apparent.

At a relatively low level of similarity two clusters occur in both trees. The first of these clusters contains a mix of species including some species of *Raoulia, Gnaphalium* and *Ewartia*. This corresponds to Clusters 8 and 9 in the Average linkage tree and Clusters 1, 2 and 3 in the single linkage tree, except for the exclusion of *Gnaphalium mackayi* and *Raoulia* sp. "M" from the cluster in the single linkage tree. The second low level cluster which is common to both dendrograms is Cluster E of the Single linkage tree. This cluster corresponds to Cluster 5 and 6 in the Average linkage tree, and contains six species of *Raoulia* and *Leucogenes leontopodium*.

Another similarity between both dendrograms at a relatively low level of similarity is the isolation of *Haastia* and *Helichrysum dimorphum*. In both dendrograms these three species are among the last taxa to be added to the tree.

The most striking difference between the two dendrograms at a low level of similarity (aside from the chaining in the single linkage tree) is the placement of the cluster of *Helichrysum intermedium, H. parvifolium, H. coralloides* and *H. depressum*. These four species form Cluster 5 in the average linkage tree, and Cluster B in the single linkage tree. In the average linkage tree Cluster 5 shows the greatest affinity with two other clusters containing species of *Raoulia* and *L. leontopodium* (Clusters 4 and 6). These combined

clusters then link with the *Gnaphalium* and *Anaphalis* cluster (Cluster E), before clustering with the remaining *Raoulia* species (Cluster C) and the *Cassinia* and *Ozothamnus* cluster (Cluster B). In the single linkage tree, Cluster B is added to the dendrogram only after all species of *Raoulia* (except *R. hectorii* and *R. tenuicaulis*) have been clustered together, and does not show the close affinity with of the species of Cluster 6 in the average linkage tree.

At a higher level of similarity eight consistent clusters occur in both dendrograms:

(1) *Haastia pulvinaris* and *H. sinclairii.*

(2) *Cassinia* and *Ozothamnus* species, excluding *O. leptophyllus.*

(3) *Helichrysum parvifolium, H. intermedium, H. depressum* and *H. coralloides.*

(4) *Raoulia glabra, R. monroi, R. hookeri, R. subsericea,* and *Leucogenes grandiceps.*

(5) *Raoulia petriensis, R. youngii*, and *Pterygopappus lawrencei.*

(6) *Raoulia grandiflora, R.* sp. "L", *Gnaphalium nitidulum*, and *Ewartia planchonii.*

(7) Three pairs of species which link together with *Ewartia catipes* to form a single cluster (a) *Raoulia cinerea* and *Rachelia glaria;*

(b) *Gnaphalium involucratum* and *Pseudognaphalium luteoalbum;*

(c) *Gnaphalium audax* and *G. traversii.*

(8) *Raoulia bryoides, R. eximia,* and *R. mammillaris.*

Other differences and similarities between the trees may be identified by examining the distribution of the genera.

Anaphalis forms a distinct cluster in the average linkage tree, but only *A. subrigida* and *A. keriensis* cluster together in the single linkage tree. None of the species in *Ewartia* associate closely with each other, although the three Tasmanian species occur in the same cluster formed at a relatively low level of similarity. *E. sinclairii* is isolated in both dendrograms, exhibiting only a weak similarity to *Helichrysum lanceolatum.*
As indicated above, both species of *Haastia* and the Tasmanian species of *Cassinia* and *Ozothamnus* form distinct generic clusters. *O. leptophyllus* clusters at a relatively low level of similarity to the Tasmanian taxa in the average linkage tree, but is isolated in the single linkage tree. Of the *Helichrysum* species only those listed in (3) above show a strong similarity to each other. *H. dimorphum* links distantly with *Haastia* in the average

linkage dendrogram, but is isolated in the single linkage dendrogram. *Helichrysum bellidioides* and *H. filicaule* both show similarities to some species of *Raoulia* subg. *Raoulia*, but do not associate closely with each other in either dendrogram. *H. filicaule* does however show a weak similarity to *H. lanceolatum* in the average linkage dendrogram. *H. lanceolatum* also exhibits a weak similarity to *E. sinclairii* in the average dendrogram, but is otherwise isolated. *Leucogenes leontopodium* and *L. grandiceps* show a greater similarity to different species of *Raoulia* than to each other in both dendrograms.

Gnaphalium audax, *G. traversii*, *G. involucratum* and *Pseudognaphalium* all show strong similarities in both dendrograms, but only slight similarities to *G. nitidulum* and *G. mackayi*. *G. nitidulum* exhibits a greatest similarity to *Raoulia grandiflora*, *E. planchonii*, and *R.* sp. "L", while *G. mackayi* occurs in a somewhat isolated position in both trees.

In both trees most species of *Raoulia* subg. *Raoulia* form one cluster from which *R.* sp. "M", *R. cinerea* and *R. haastii* are excluded. *R. cinerea* shows greatest similarity to *Rachelia glaria*, while *Raoulia* sp. "M" occurs in a relatively isolated position in both dendrograms. *Raoulia haastii* clusters most closely to the only close cluster between members of R*aoulia* subg. *Psychrophyton* (i.e. *R. bryoides*, *R. eximia*, and *R. mammillaris*). Of the other species in R*aoulia* subg. *Psychrophyton*, *R. grandiflora* *R.* sp. "L" and *R. youngii* occur in same cluster, but do not show strong similarities to each other, while *R. subulata* and *R. hectorii* cluster with *Leucogenes leontopodium* in both trees.
Raoulia petriensis and *Pterygopappus* show a high level of similarity in both dendrograms.

Principal Coordinate Analysis

The first three axes of the PCoA explain 80% of the variation in the data set, with each axis individually explaining 39.6%, 24.5% and 16.0% respectively.

No clearly demarcated groups are readily recognisable in the PCoA plots (Figure 2.12), but a number of species are consistently closely placed. For example, *Raoulia cinerea* and *Rachelia glaria* are closely placed on all three axes, as are *Helichrysum depressum*, *H. parvifolium*, *H. intermedium*, and *H. coralloides*. Other such examples include:

Cassinia and Ozothamnus; *Anaphalis trinervis* and *Ewartia catipes*; *Gnaphalium involucratum* and *Pseudognaphalium*; *G. nitidulum* and *Raoulia grandiflora.*

The graphs also highlight the isolation of some species. For example *Helichrysum filicaule* shows only a weak association with *Raoulia monroi* on the plot of the first and second axes, but is otherwise quite isolated. *Helichrysum dimorphum* is also isolated, not consistently occurring very closely to any one particular species.

The PCoA plots also illustrate the overlap between the clusters formed in the average linkage dendrogram above a similarity of 0.6695. Nearly all the clusters formed at this level show some degree of overlap in the PCoA plots (Figure 2.12).

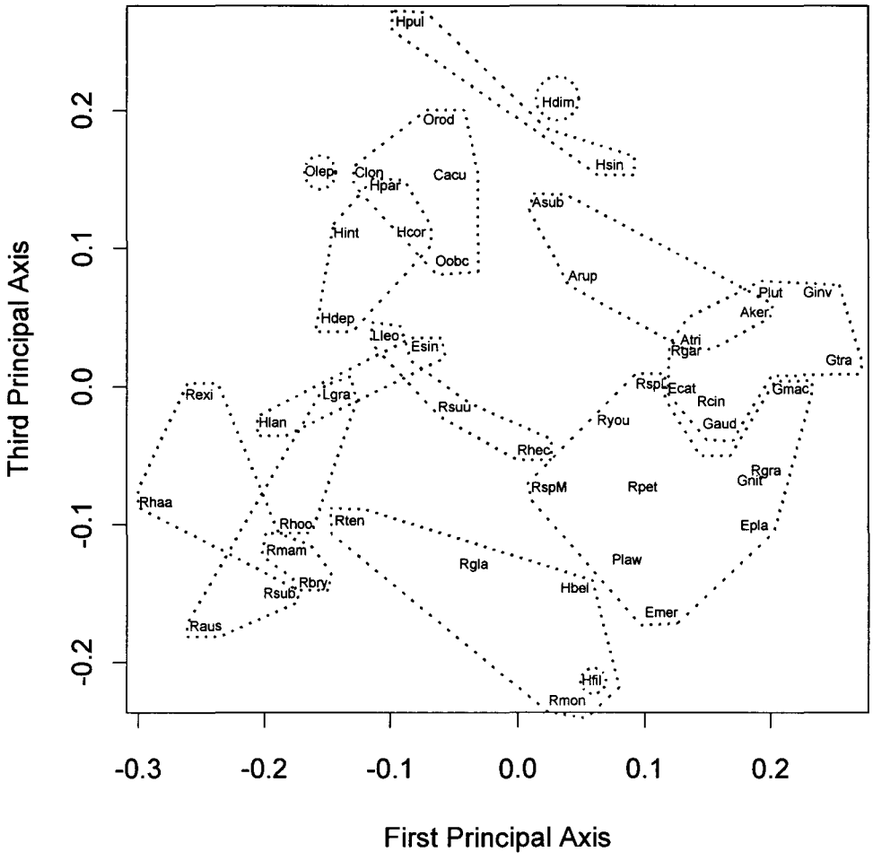
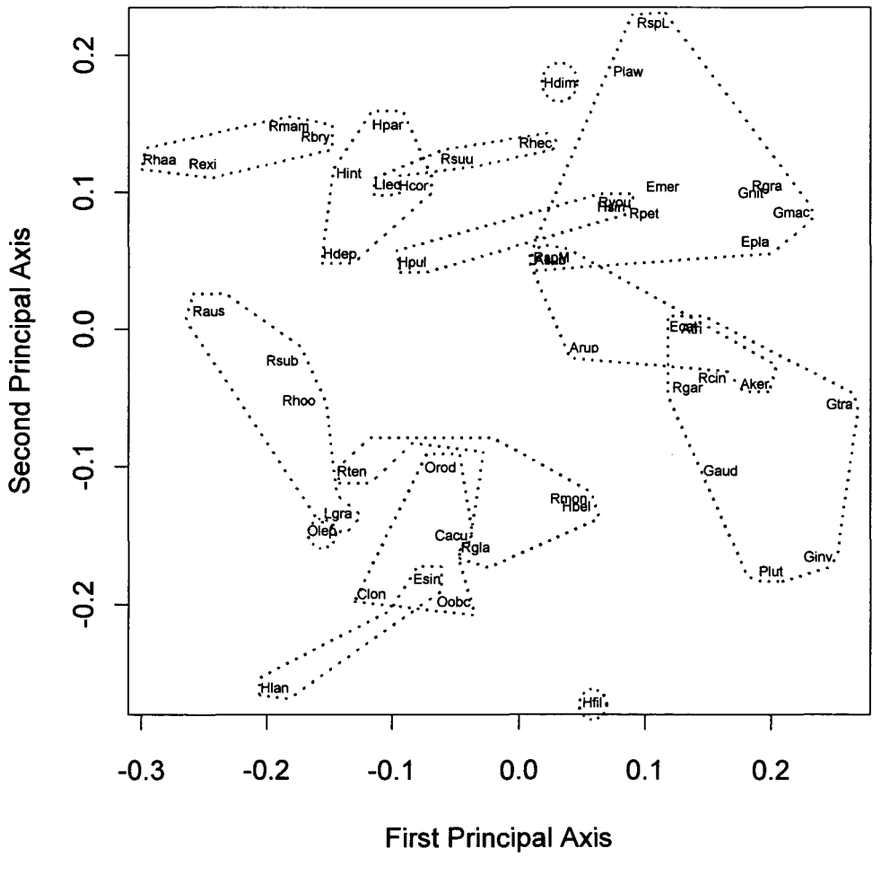


Figure 2.12: Scatter plot of the study species on the first three Principal coordinate analysis axes generated from Gower's General Coefficient of Similarity based upon their stem anatomy. The groupings indicate clusters formed in the Average Linkage dendrogram above a similarity of 0.6695.



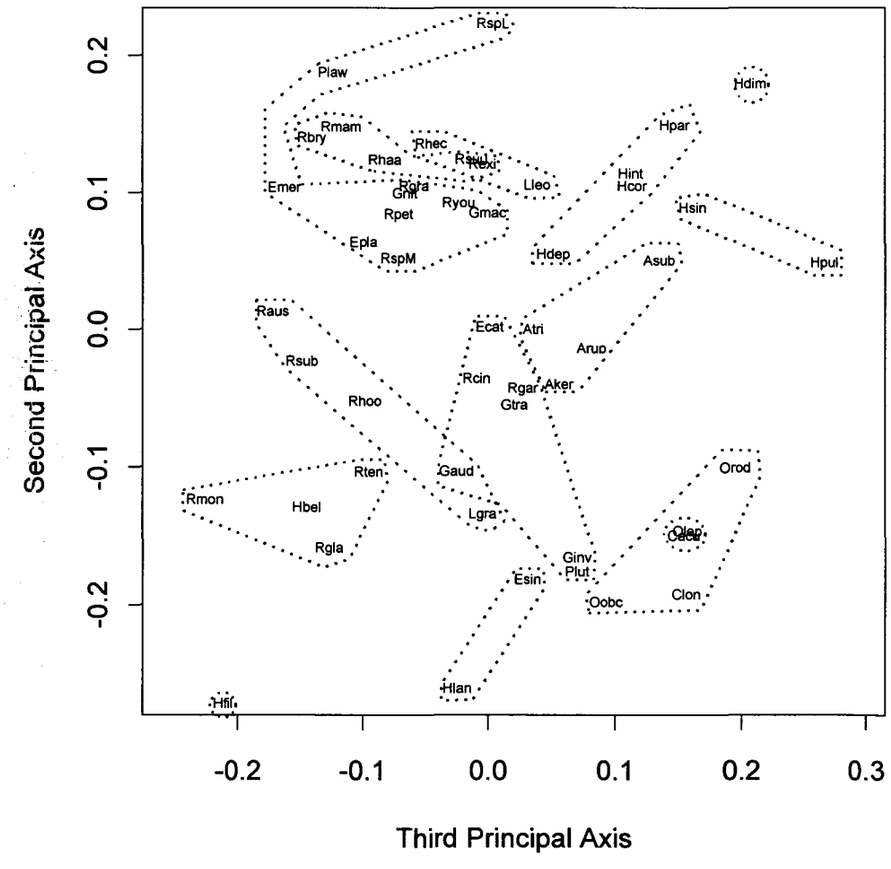


Plate 1: Pith cell end walls and thickening

A: *Helichrysum coralloides*: Pith cells evenly lignified, with smooth, pitted end walls (arrows) (MPS). Scale = 50 μm.

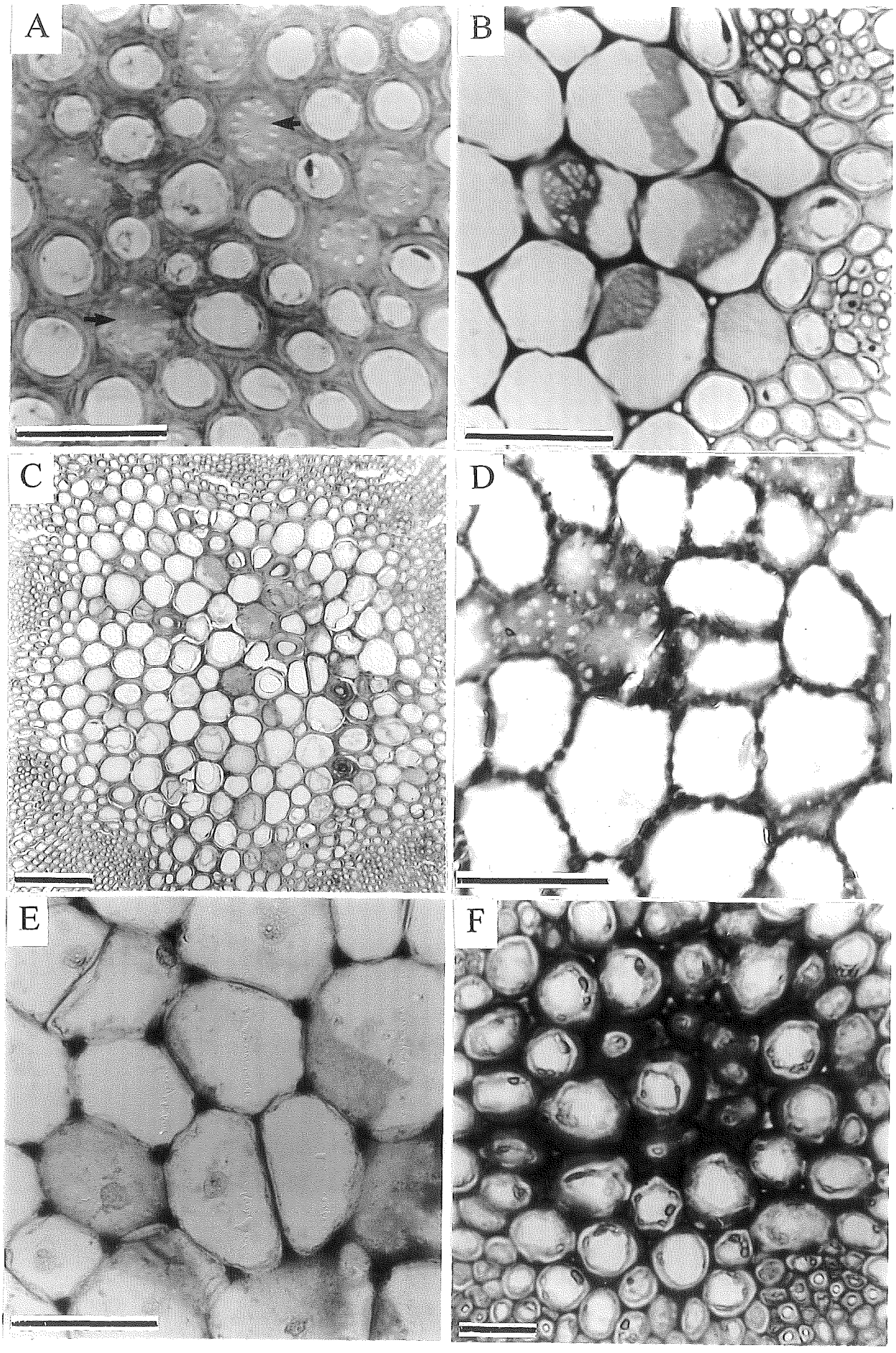
B: *Ozothamnus leptophyllus*: Pith cells with fibrous end walls (MSS). Scale = 50 μm.

C: *Leucogenes grandiceps*: Pith cells with evenly lignified walls, some cells with walls massively thickened (MSS). Scale = 100 μm.

D: *Gnaphalium audax*: Pith cells with "warty" collenchymatous type thickening (Tip). Scale = 50 μm.

E: *Anaphalis rupestris*: Pith cells with collenchymatous thickening at the cell corners (Tip). Scale = 50 μm.

F: *Raoulia subsericea*: Pith cells with wide spread collenchymatous thickening (Tip). Scale = 100 μm.


A
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F

Plate 2: Intercellular pith spaces and primary xylem.

A: *Gnaphalium involucratum*: Intercellular pith spaces unfilled (MPS). Scale = 50 μm.

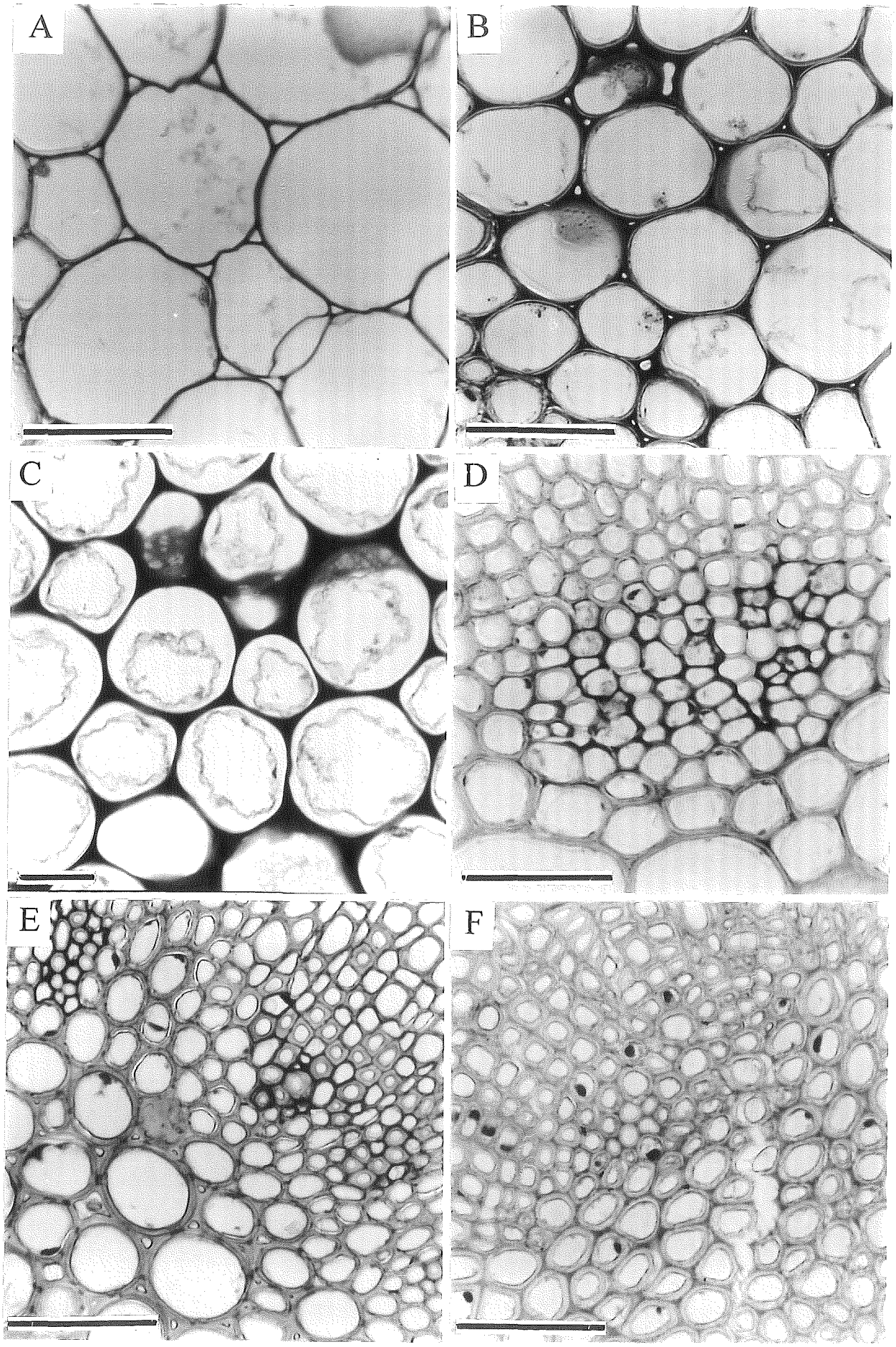
B: *Ewartia planchonii*: Intercellular pith spaces partially and completely filled (MSS). Scale = 50 μm.

C: *Leucogenes grandiceps*: Intercellular pith spaces filled (MPS). Scale = 100 μm.

D: *Pseudognaphalium luteoalbum*: Some cells in primary xylem unlignified (MSS). Scale = 50 μm.

E: *Helichrysum intermedium*: Some cells in primary xylem unlignified (MSS). Scale = 50 μm.

F: *Pterygopappus lawrencei*: All cells in primary xylem lignified (MSS). Scale = 50 μm.


A
B
C
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E
F

Plate 3: Vessel grouping and growth rings.

A: *Haastia pulvinaris*: Tangential vessel aggregations (MSS).
Scale = 100 μm.

B: *Helichrysum lanceolatum*: Radial vessel aggregations (MSS).
Scale = 100 μm.

C: *Cassinia longifolia*: Clustered vessel aggregations (MSS).
Scale = 100 μm.

D: *Raoulia petriensis*: Solitary vessels (MSS). Scale = 50 μm.

E: *Raoulia haastii*: Type 2 growth ring (MSS). Scale = 50 μm.

F: *Helichrysum depressum*: Type 5 growth ring (MSS). Scale = 50 μm.

Pith located to the left in all photographs.

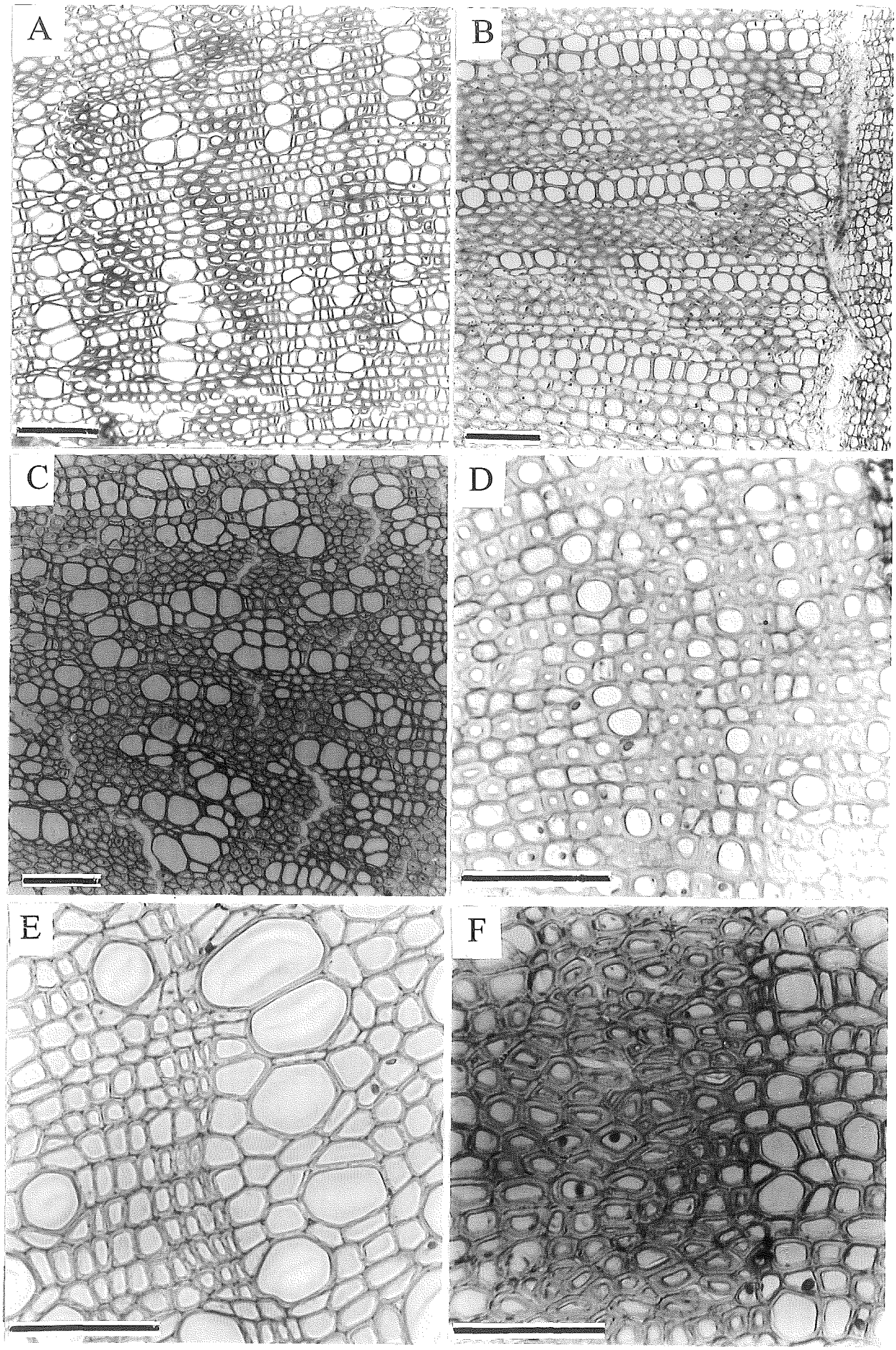

A
B
C
D
E
F

Plate 4: Rays and axial parenchyma

A: *Raoulia tenuicaulis*: Medullary rays (MSS). Scale = 100 μm.

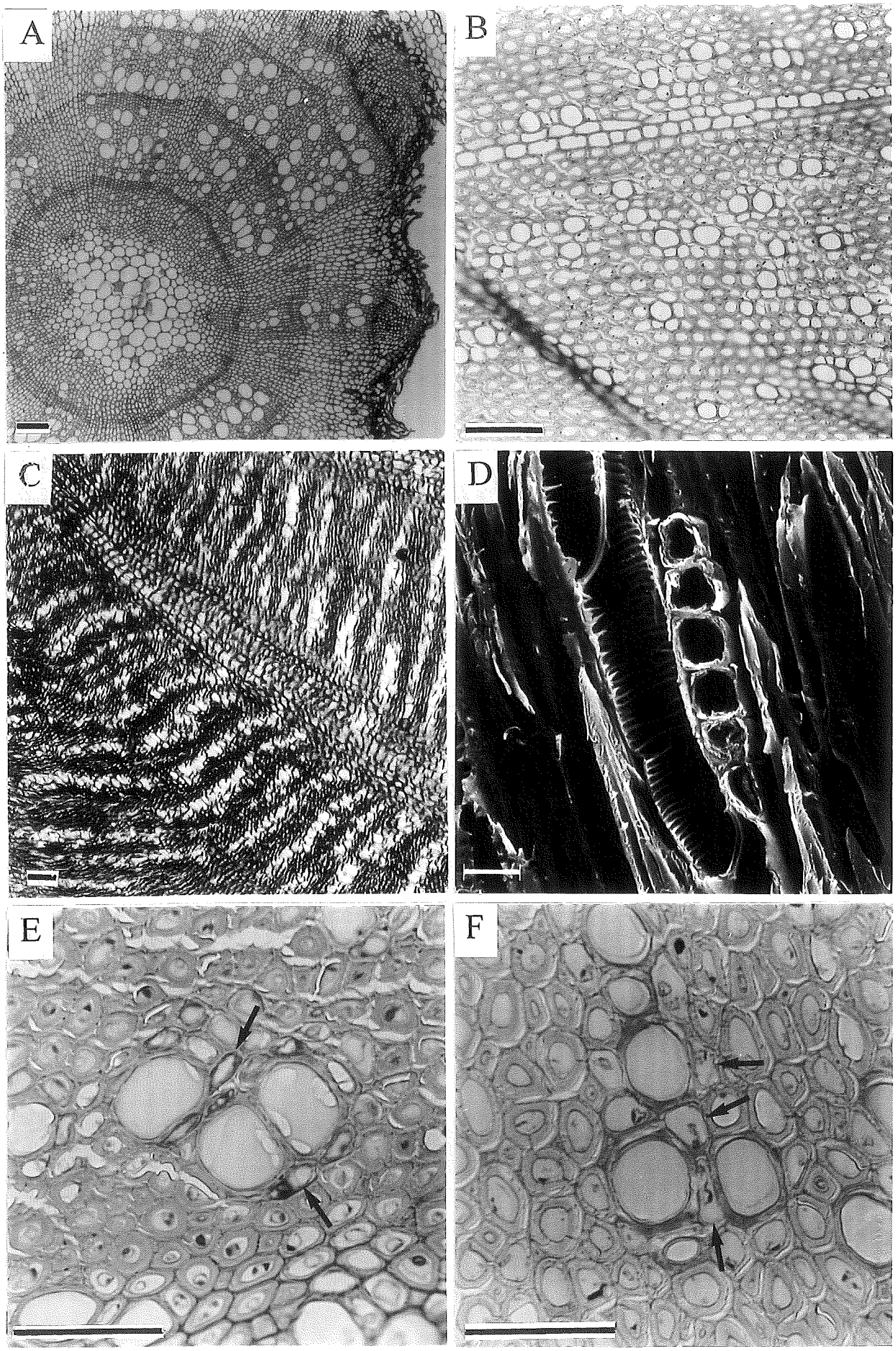
B: *Helichrysum lanceolatum*: Narrow multiseriate ray (MSS).
Scale = 100 μm.

C: *Raoulia eximia*: Wide multiseriate ray (MSS). Sledge section.
Scale = 100 μm.

D: *Cassinia longifolia*: Uniseriate ray (MSS). TLS under SEM.
Scale = 10 μm.

E: *Cassinia aculeata*: Parenchyma associated with vessels (arrowed)
(MSS). Scale = 50 μm.

F: *Ozothamnus obcordatus*: Parenchyma associated with vessels (arrowed)
(MSS). Scale = 50 μm.


A
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F

Plate 5: Anomalous cambium activity and phloem fibres.

A: *Raoulia eximia*: Anomalous secondary growth in TS under SEM (MSS). Scale = 100 μm.

B: *Raoulia bryoides*: Anomalous secondary growth. Note old leaves to bottom left of pith (arrow) (MSS). Scale = 100 μm.

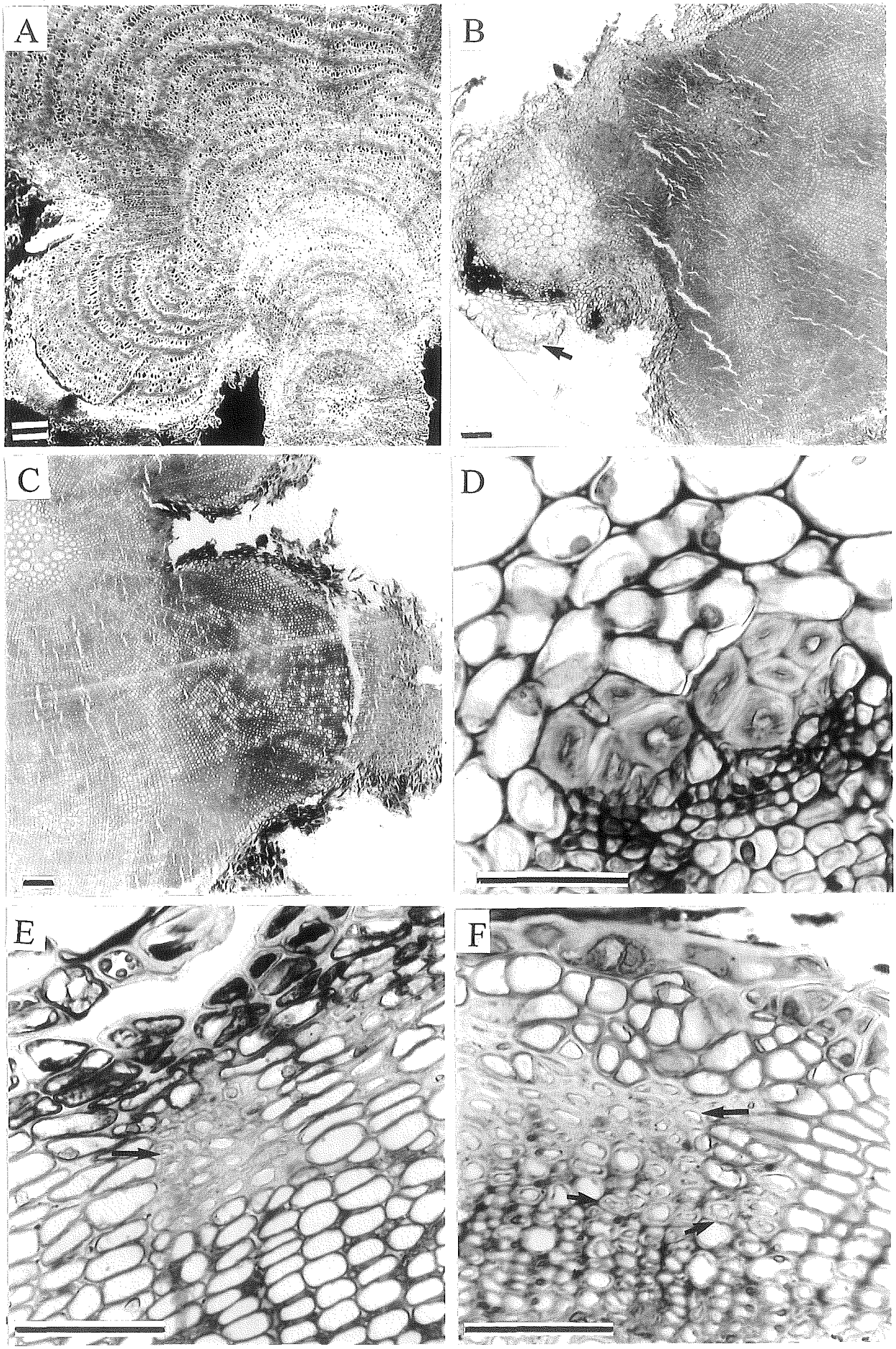
C: *Raoulia mammillaris*: Anomalous secondary growth. Note fibre cap at end of lobe (MSS). Scale = 100 μm.

D: *Haastia pulvinaris*: Phloem fibres with massively thickened walls (MPS). Scale = 50 μm.

E: *Raoulia haastii*: Phloem fibres arranged in a mass (MSS) (arrow). Scale = 50 μm.

F: *Raoulia australis*: Early phloem fibres arranged in a mass (long arrow), later fibres clearly separate (short arrows) (MSS). Scale = 50 μm.

Pith to bottom in A, D, E, and F.


A
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C
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F

Plate 6: Casparian strip

A: *Anaphalis rupestris*: (MPS). Scale = 50 μm.

B: *Ewartia catipes*: (MPS). Scale = 100 μm.

C: *Gnaphalium traversii*: (MPS). Scale = 50 μm.

D: *Haastia pulvinaris*: (Tip). Scale = 100 μm.

E: *Helichrysum coralloides*: (MPS). Scale = 100 μm.

F: *Raoulia* sp. "L": (MPS). Scale = 100 μm.

Pith to bottom in all photos.

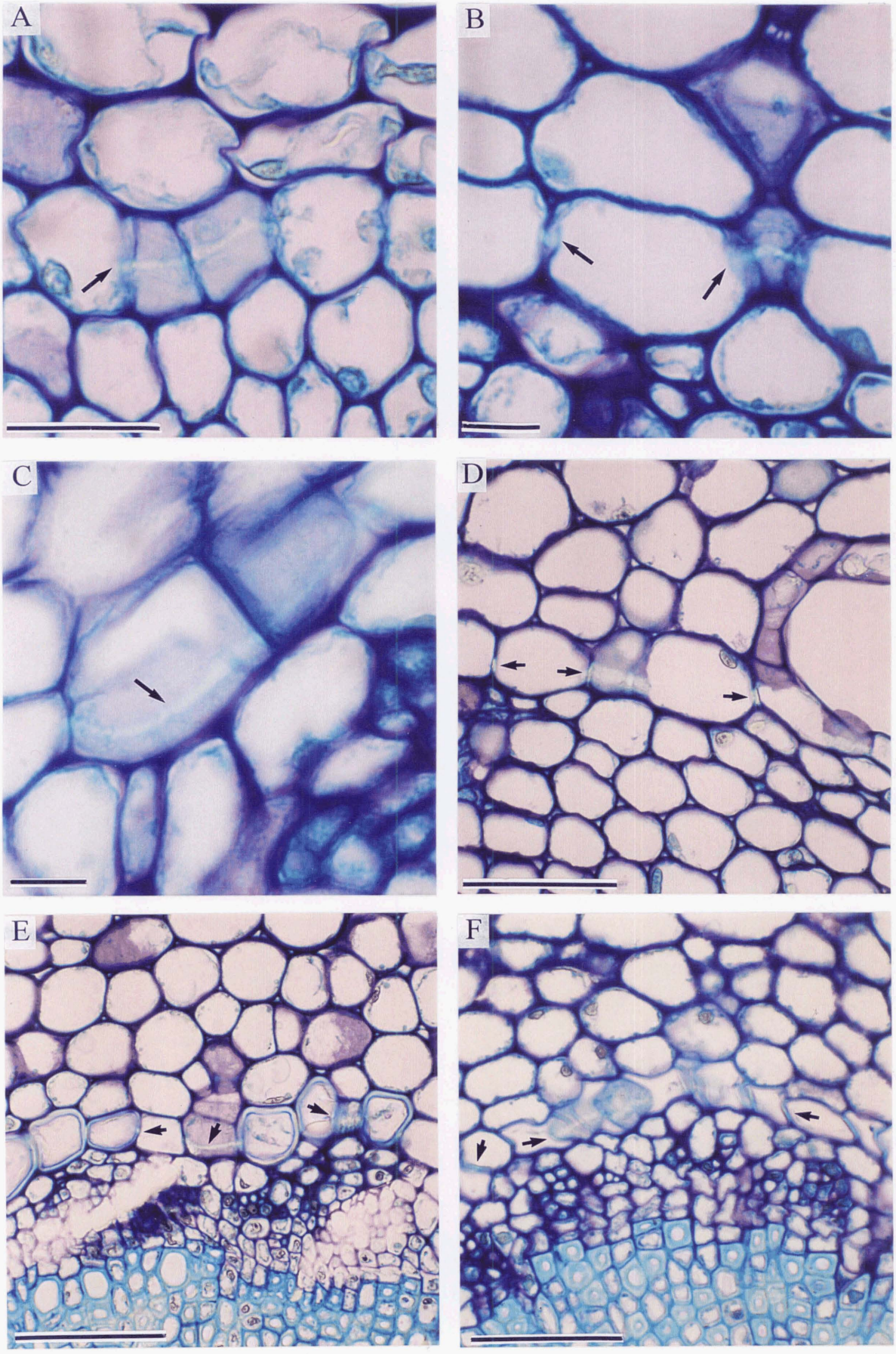
A
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Plate 7: Endodermis thickening and resin canals.

A and B: *Helichrysum lanceolatum*: Outer tangential endodermis walls thickened (arrow) (Bright Field), and birefringent (Polarised). (MPS). Scale = 50 μm.

C: *Raoulia australis*: Radial and outer tangential walls thickened (MPS). Scale = 50 μm.

D: *Ozothamnus leptophyllus*: All endodermis walls thickened (MPS). Scale = 50 μm.

E: *Haastia pulvinaris*: Resin canal (r) in cortex (Tip). Scale = 50 μm.

F: *Haastia sinclairii*: Resin canals (r) in cortex (surrounded by dark stained cells) (Tip). Scale = 50 μm.

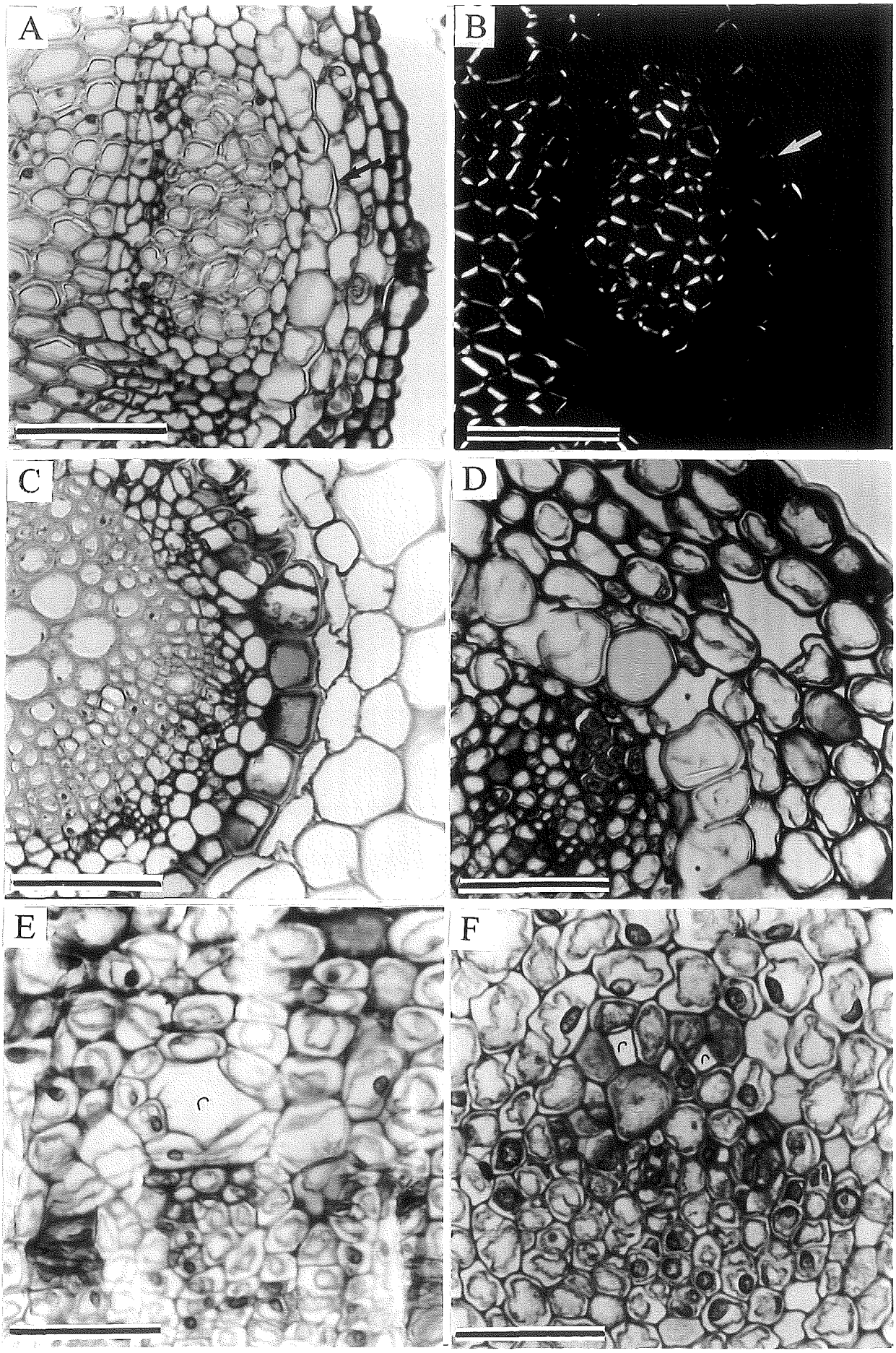
A
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Plate 8: Type of cortex.

A: *Ewartia planchonii:* Homogeneous cortex (Tip). Scale = 100 μm.

B: *Ozothamnus rodwayi*: Homogeneous cortex (MPS). Scale = 100 μm.

C: *Helichrysum dimorphum*: Large outer cells (MPS). Scale = 50 μm.

D: *Helichrysum parvifolium*: Large outer cells (MPS). Scale = 50 μm.

E: *Ozothamnus obcordatus*: Large inner cells (Tip). Scale = 50 μm.

F: *Helichrysum filicaule*: Large inner cells (MPS). Scale = 50 μm.

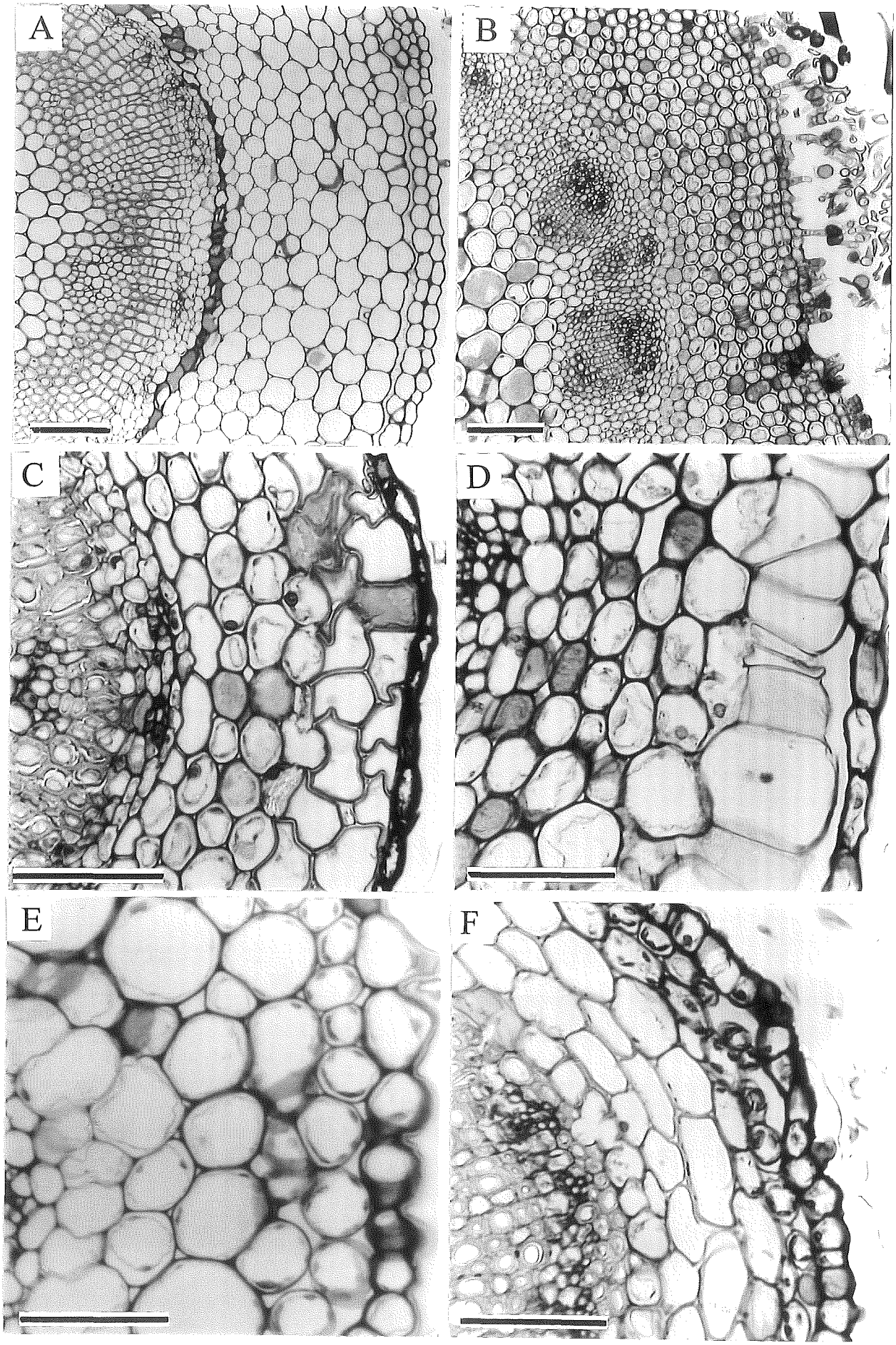
A
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Plate 9: Lignified cortex cells and type of stomata.

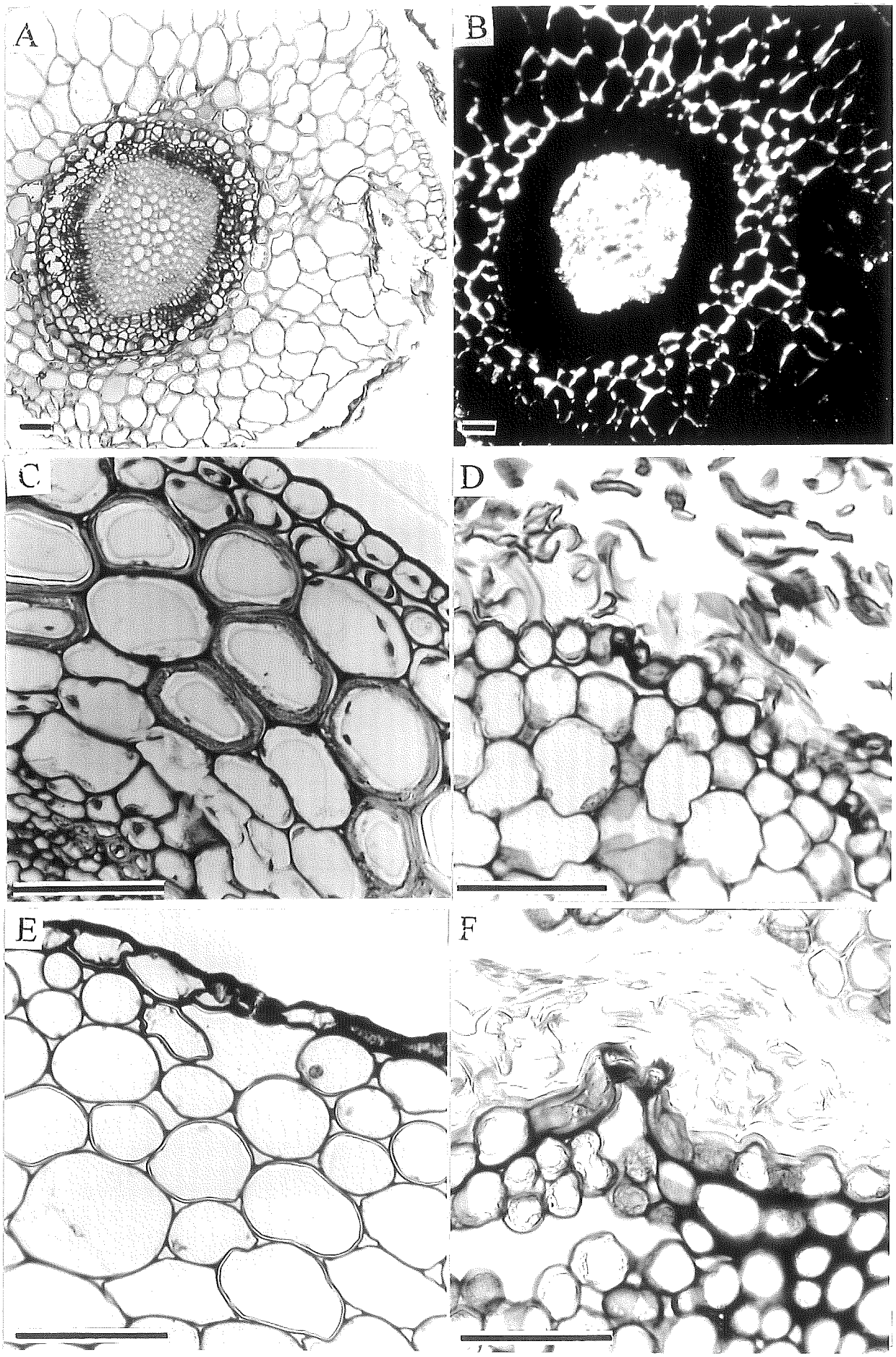
A and B: *Raoulia subsericea*: All cortex cells lignified, under bright field and polarised light (MPS). Scale = 100 μm.

C: *Raoulia tenuicaulis*: Scattered lignified cells with thickened walls (MPS). Scale = 50 μm.

D: *Raoulia monroi*: Stomata level with epidermis (Tip). Scale = 50 μm.

E: *Gnaphalium audax*: Stomata level with epidermis (Tip). Scale = 50 μm.

F: *Ozothamnus leptophyllus*: Raised stomata (Tip). Scale = 50 μm.


A
B
C
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E
F

Plate 10: Cortex spaces and aerenchyma.

A: *Gnaphalium involucratum*: Prominent spaces at the cell corners (MPS). Scale = 50 μm.

B: *Raoulia* sp. "M": Large space directly under the epidermis (Tip). Scale = 50 μm.

C: *Ewartia catipes*: Large space under the epidermis (MPS). Scale = 50 μm.

D: *Ozothamnus leptophyllus*: Large space in cortex with two layers of cell between space and cortex (Tip). Scale = 100 μm.

E: *Ewartia meredithiae*: Aerenchymatous cortex spaces (MSS). Scale = 100 μm.

F: *Ewartia meredithiae*: Aerenchymatous cortex spaces (MSS). Scale = 50 μm.

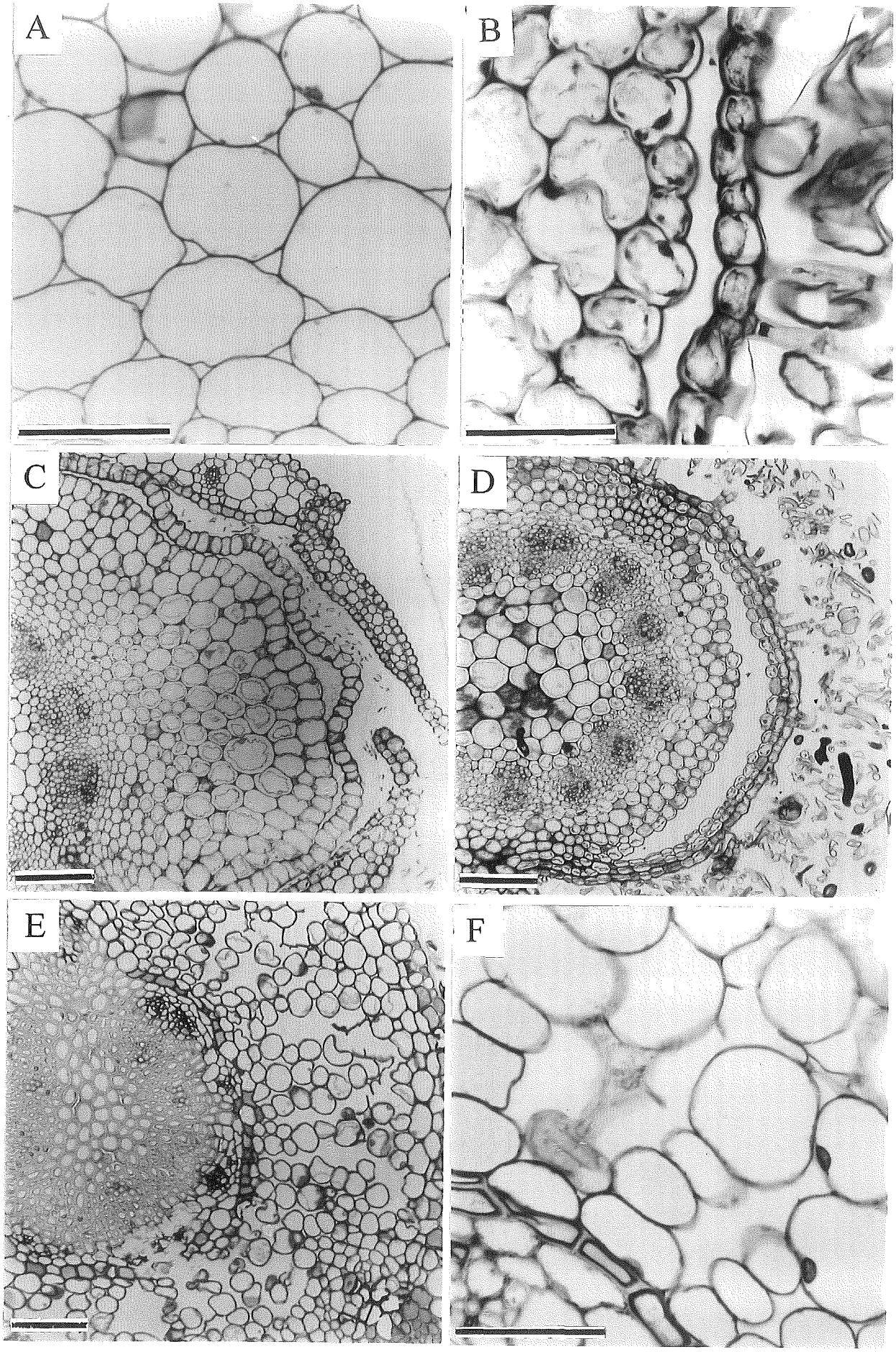

A
B
C
D
E
F

Plate 11: Cuticle ridges and biseriate hairs.

A: *Raoulia tenuicaulis*: Type A ridges (i = Tip; ii = MPS). Scale = 50 μm.

B: *Raoulia subsericea*: Type B ridges on leaf sheath (adaxial surface to top) (MPS). Scale = 50 μm.

C: *Anaphalis subrigida*: Type C ridges (MPS). Scale = 50 μm.

D: *Raoulia monroi*: Striations in the cuticle (Sheath - abaxial surface to top) (Tip). Scale = 50 μm.

E: *Cassinia longifolia*: Biseriate hair without swollen terminal cells (Tip). Scale = 50 μm.

F: *Cassinia aculeata*: Biseriate hair with swollen terminal cells (Tip). Scale = 50 μm.

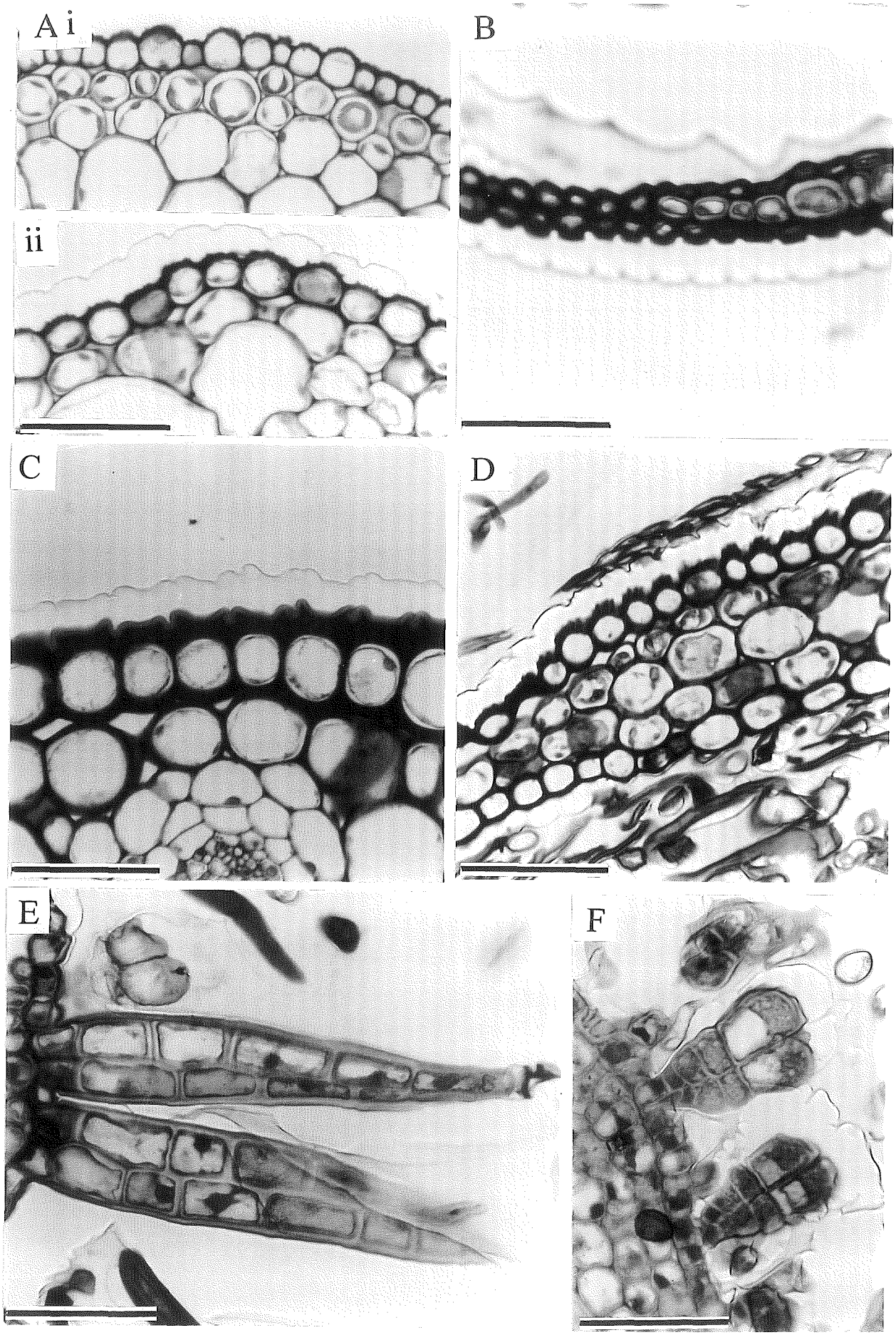

A i
ii
B
C
D
E
F

Plate 12: Outer layer at maturity and periderm.

A: *Raoulia subsericea*: Endodermis as the outer layer at maturity (MSS).
Scale = 50 um.

B: *Raoulia glabra*: Endodermis as the outer layer at maturity (MSS).
Scale = 50 μm.

C: *Ozothamnus leptophyllus*: Periderm located in the outer phloem (MSS).
Scale = 100 μm.

D: *Haastia sinclairii*: Periderm located in the outer cortex (MSS).
Scale = 100 μm.

E: *Helichrysum depressum*: Sequential periderm layers (MSS).
Scale = 100 μm.

F: *Helichrysum depressum*: Detail of periderm layers (MSS).
Scale = 50 μm.

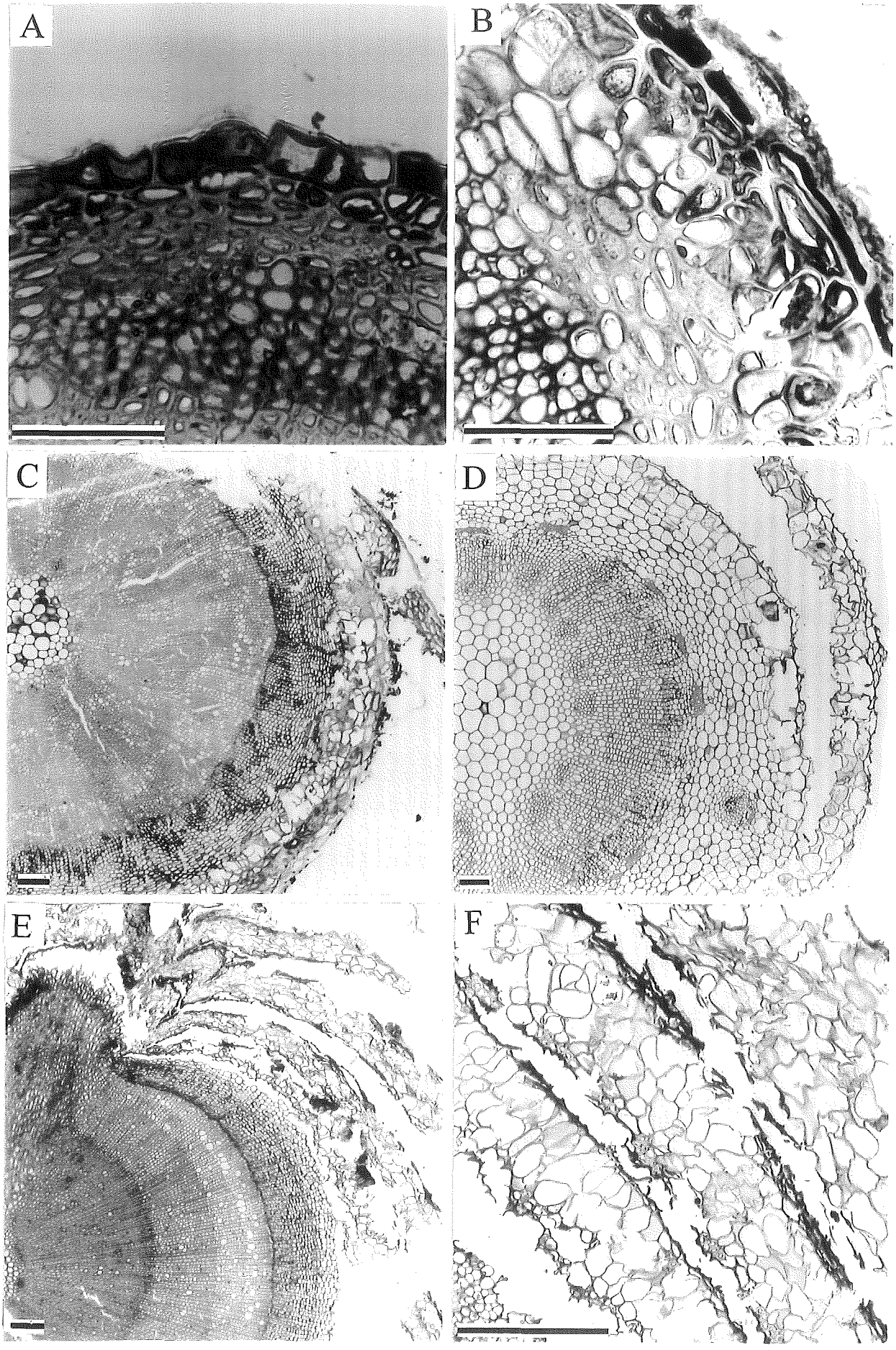

A
B
C
D
E
F

Plate 13: Nodal anatomy.

A: *Ozothamnus leptophyllus*: Variable number of leaf gaps and traces. Two leaf traces visible in the leaf to top left (lateral arrowed), one vein in fully detached leaf (Tip). Scale = 100 μm.

B: *Raoulia hectorii*: Trilacunar node (Tip). Scale = 100 μm.

C: *Anaphalis rupestris*: Multilacunar node (MPS). Scale = 100 μm.

D: *Anaphalis rupestris*: Detail of lateral traces (MPS). Note the two endodermal layers surrounding the traces. Scale = 100 μm.

E: *Helichrysum lanceolatum*: Trilacunar node. Note endodermis surrounding leaf traces (Tip). Scale = 100 μm.

F: *Raoulia bryoides*: Unilacunar node (MPS). Scale = 100 μm.

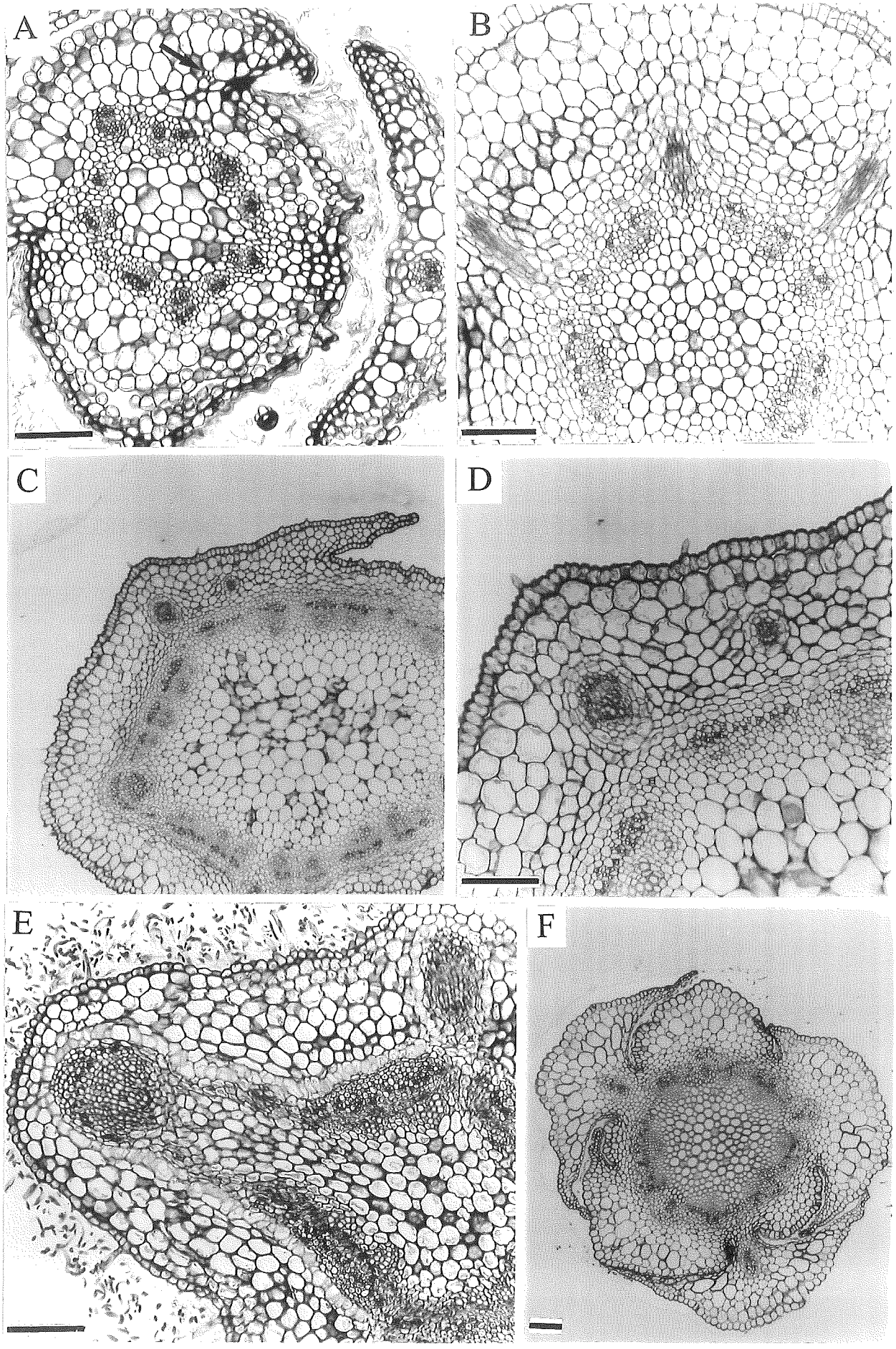

A
B
C
D
E
F

Plate 14: Number of leaf sheath veins and leaf sheath sclerenchyma.

A: *Pseudognaphalium luteoalbum*: Lateral vein dividing in cortex (MPS). Scale = 100 μm.

B: *Haastia sinclairii*: Lateral veins dividing in cortex (arrowed) (Tip). Note resin canals associated with the leaf traces. Scale = 100 μm.

C: *Cassinia aculeata*: Veins dividing in the leaf sheath (arrowed) (Tip - adaxial surface to top). Scale = 100 μm.

D: *Raoulia subsericea*: Sclerified mesophyll cells (adaxial to top right) (MPS). Scale = 50 μm.

E: *Gnaphalium nitidulum*: Adaxial mesophyll sclerified (Tip). Scale = 100 μm.

F: *Raoulia* sp. "L": Adaxial epidermis sclerified (Tip). Scale = 100 μm.

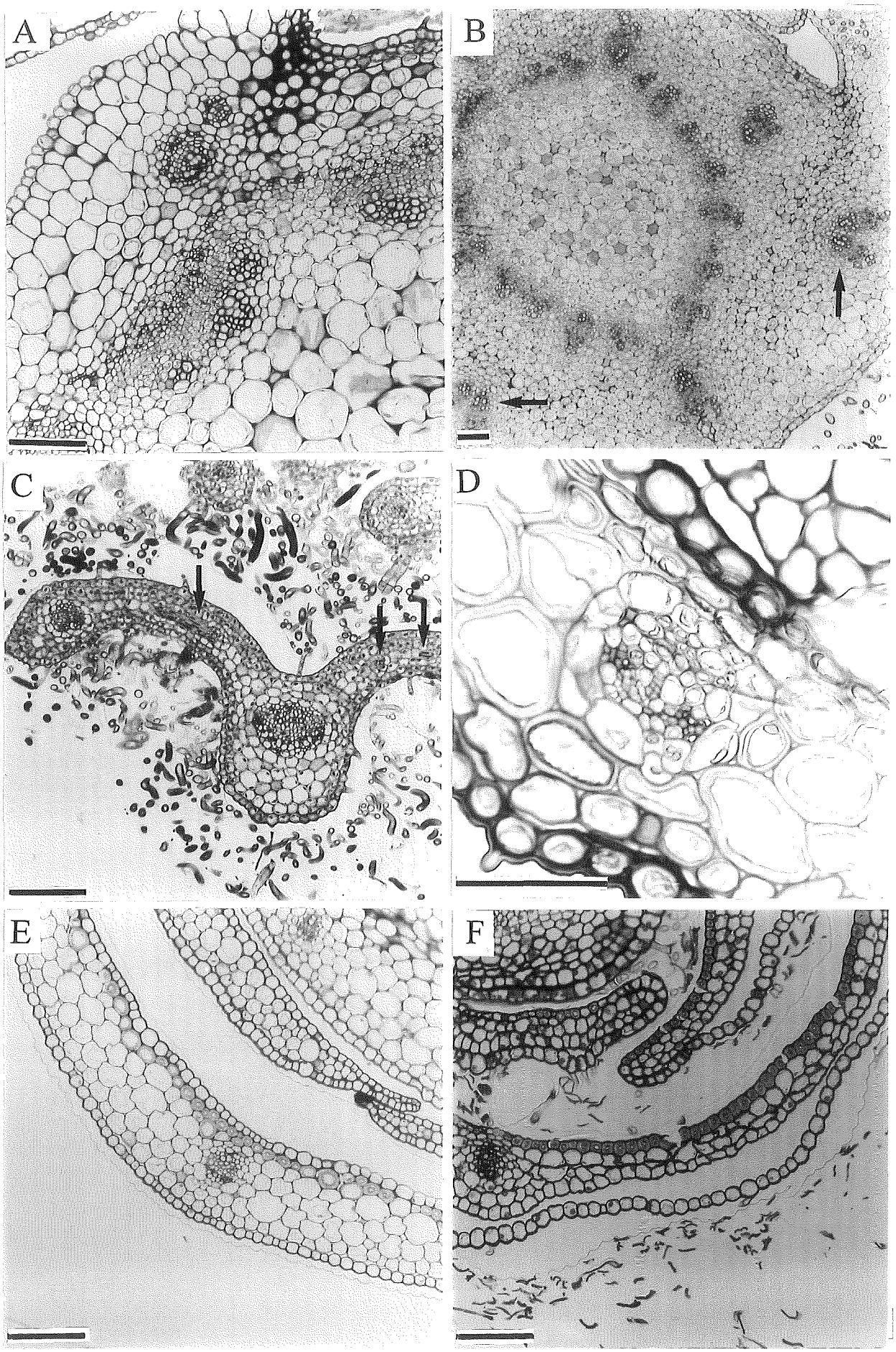

A
B
C
D
E
F

Plate 15: Sclerenchyma bundle caps in veins of leaf sheath.

A: *Helichrysum coralloides*: Abaxial sclerenchyma cap (Tip). Scale = 100 μm.

B: *Helichrysum lanceolatum*: Abaxial sclerenchyma cap (arrowed), and sclerified bundle sheath cells (most cells in sheath) (Tip). Scale = 50 μm.

C: *Leucogenes leontopodium*: Abaxial (to top) and adaxial sclerenchyma cap (Tip). Scale = 50 μm.

D: *Ewartia meredithiae*: Sclerenchymatous cylinder (Tip). Scale = 50 μm.

E: *Raoulia grandiflora*: adaxial sclerenchyma caps in main and lateral veins (MPS). Scale = 100 μm.

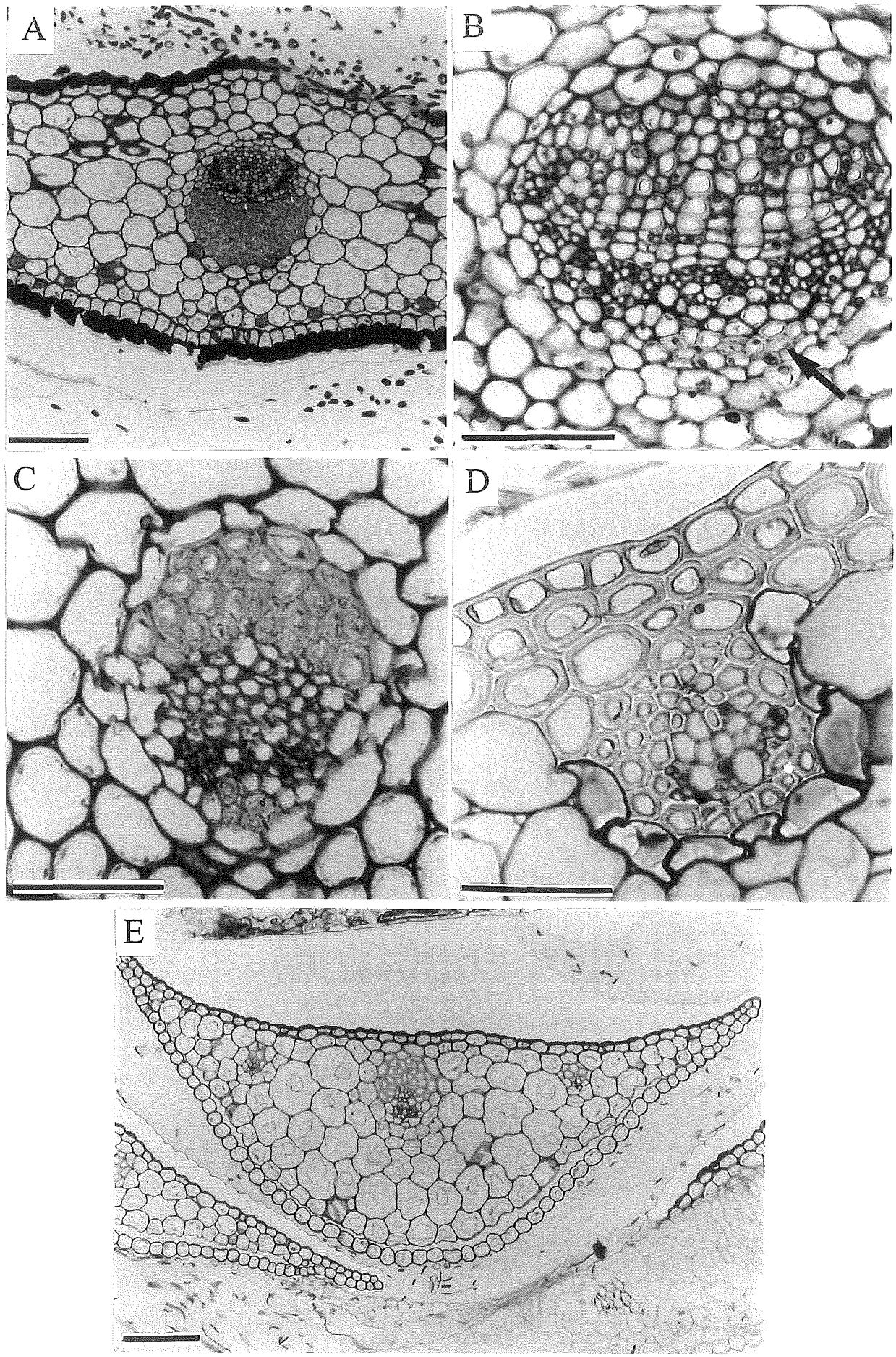

A
B
C
D
E

Plate 16: Crystals and starch grains.

A: *Gnaphalium mackayi*: Silica body in the cortex of MPS section under bright field (i) and polarised light (ii). Scale = 10 μm.

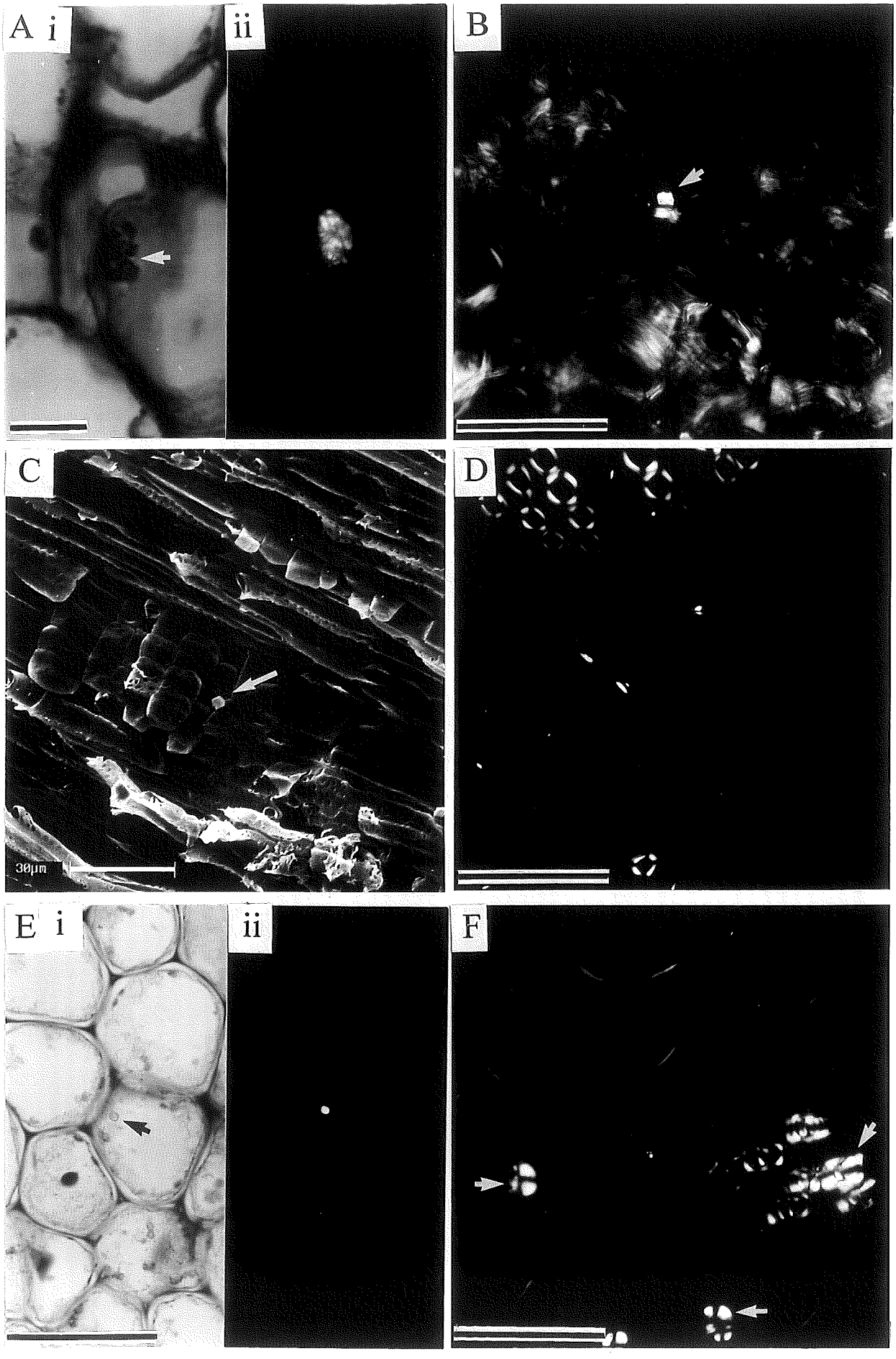
B: *Helichrysum coralloides*: Rhomboidal crystal located in the periderm. Sledge section of MSS. Scale = 50 μm.

C: *Helichrysum intermedium*: Square crystal (arrowed) located in LS using SEM. Scale = 30 μm.

D: *Cassinia longifolia*: Rod shaped crystals in the pith under polarised light. Scale = 50 μm.

E: *Cassinia longifolia*: Spherical crystals in the pith as seen in bright field (i) and polarised light (ii). Scale = 50 μm.

F: *Haastia pulvinaris*: Starch grains in the cortex (arrowed). Scale = 50 μm.


A i
ii
B
C
30µm
D
E i
ii
F

2.4 Discussion

Many of the early anatomical investigations sought to understand the anatomical characteristics of plants in terms of adaptations to their environment (e.g. Haberlandt, 1914). In the New Zealand Inuleae the early works of Hauri (1917) and Foweraker (1917) are mainly physiologically oriented, interpreting features such as the strong precocious cork development and a prominently thickened endodermis as adaptations to a cushion or mat growth form and a xeric habitat. Similarly, Betts (1920b) interpreted the large cells in the cortex of *Gnaphalium traversii* as water storage tissue. Other features of the stem, and in particular the wood, have also been interpreted as functional adaptations to the environment. For example, features which have been given functional interpretations are, the development of aerenchyma in very wet habitats (Solereder, 1908), and the grouping and narrowing of vessels in xeric habitats (Carlquist, 1988). In a series of works spanning 30 years, Metcalfe (1950; 1954; 1959; 1983) defends the systematic use of characters which are adaptations to environmental conditions on the grounds that such characters are "in all probability, primarily hereditary and secondarily of ecological value" (Metcalfe, 1954). As evidence he points out that such adaptations are limited by the hereditary potential of the species, so that any species of different phylogenetic origin will usually achieve adaptations to the same environment in different ways, so that not all adaptive features will be found in any one species (Metcalfe, 1954; 1983). In this study all characters are regarded as being under genetic control, including those which may have evolved as an adaptation to a particular environmental condition. For example, the development of an aerenchymatous cortex in *Ewartia meredithiae* is undoubtedly an adaptation to environmental conditions, as this species is typically found growing in saturated soils (pers. comm. J.M. Ward). That this development is under some degree of genetic control is suggested by the absence of aerenchyma in the samples of other species, in particular *R. subulata* from Princess Bath and *G. mackayi*, which were collected from saturated sites with saturated soil conditions. However, the findings of Kawase (1979) and Kawase and Whitmoyer (1980) that the development of aerenchmya in other species of Compositae could occur in as little as two days, indicates that this can be a very plastic character. The presence of an aerenchymatous cortex has also been used in a cladistic and biogeographical study of the *Lucilia* group (Gnaphaliinae, Inuleae) of South America (Anderberg and Freire, 1991). The genetic basis of the features such as aerenchmya

development in *E. meredithiae*, could be explored further through the examination samples from glasshouse populations grown under varying conditions.

Metcalfe (1983c) argues that many characters which vary in a given species in response to different growing conditions are usually quantitative and comparatively minor. This has previously also been suggested by Carlquist (1960b), who found quantitative, but not qualitative differences between samples of the same species he examined in the tribe Cichorieae (Compositae). In addition, Stern and Greene (1958) found that qualitative characters matured much more quickly than quantitative characters. Thus the use of mainly qualitative characters in this study potentially minimised the amount of environmentally related variation. In addition, the examination of samples from two geographical locations for each species allowed characters which were particularly variable to be eliminated from the data set (e.g. cuticle thickness). Given the result of Stern and Green, the use of qualitative characters may also have helped to reduce variation in characters due to the samples being of different ages. Furthermore, the examination of three stages of stem development was an important part of character selection, since it allowed the identification of characters that varied with age. For example, the nature of the pith was very variable in the young stem, but showed good specific characteristics in the older stem. Thus the appearance of the pith in young stems was eliminated from analysis. However, some variation between samples of the same species for the characters that were analysed was still present (e.g. the presence of Casparian strips in only one sample of *Ewartia catipes*). This variation should be explored further through the examination of more samples from wild and glasshouse populations.

The presence or absence of growth rings has commonly been assumed to be of little taxonomic significance because of the belief that they are formed in response to environmental conditions, rather than being under genetic control (Chalk, 1983). However, Carlquist (1961a, p. 49) states that the "presence of growth rings and their width may be used systematically." This statement was supported by findings of Tomlinson and Craigehead (1972), who, on the basis of a range of tropical tree species, conclude that (for their study species at least) "the ability to develop growth rings is primarily determined by the genetic make-up of the individual species". Furthermore, even in those species that did produce growth rings they were unable to find a simple correlation between climate, phenology and the development of growth rings. In this study it is assumed that the ability

to produce growth rings is under genetic control in the New Zealand Inuleae for three reasons. Firstly, all the samples were collected from the field and were therefore subject to marked seasonal changes in day length and temperature, and possibly water availability. Secondly, in some instances specimens of different species were collected from the same habitat and location, for example, of the species collected from the same habitat on Banks Peninsula growth rings were observed in *Helichrysum lanceolatum*, but not in *H. bellidioides, H. filicaule* or *Raoulia monroi*. Similarly, growth rings were present in *R. eximia*, but not in *R. mammillaris*, even though one of the samples for each of these species was collected from the same habitat and locality at Mt. Plenty (Torlesse Range). Thus the lack of growth rings can not be credibly attributed to a constant growing environment. Finally, in the species in which growth rings were observed, different elements in the secondary xylem were responsible for delimiting the growth rings. These three points suggest that the presence or absence of growth rings, and the type of growth rings are genetically controlled, and thus available for systematic purposes in the New Zealand Inuleae. The most notable groups suggested by the growth rings are those in *Raoulia* and *Helichrysum*. In all species of *Raoulia* subg. *Psychrophyton,* except *R. eximia*, growth rings were absent. This contrasts with *Raoulia* subg. *Raoulia* in which growth rings were observed in all species except *R. cinerea, R.* sp. "M", and *R. monroi*. In the woody *Helichrysum* species the type of growth ring distinguishes the three whipcord species and *H. dimorphum* (which are characterised by changes in vessel abundance and/or size, i.e. type 3 or 4) from *H. depressum* (which has an increase of thick walled cells in the late wood - type 5) and *H. lanceolatum* (which only exhibits variation in imperforate tracheary elements - type 1).

The term anomalous secondary growth encompasses a range of cambial variants that differ from the normal vascular cambium found in most dicotyledons. The occurrence of anomalous secondary growth is taxonomically restricted (Metcalfe, 1983a) and as such is of potential systematic value. Carlquist (1988) lists nine types of "cambial variants", at least three of which have been reported in the Compositae. Adamson (1934) observed interxylary (or included) phloem in species of five South African genera of the Inuleae. One of these genera, *Lachnospermum*, was included in the Gnaphaliinae by Merxmüller *et al.* (1977). In these genera Adamson found that a unidirectional cambium developed in the pericycle which produced, to the inside, a band of phloem and xylem embedded in lignified ground tissue. Adamson suggested that this feature may have arisen to allow a shrubby

habit to be achieved by plants derived from a herbaceous ancestor that lacked a normal cambium. Interxylary cork was reported by Moss (1940) in a number of *Artemisia* species, and in *A. herba-alba* by Ginzburg (1963). Moss suggested that the occurrence of interxylary cork in these plants had a functional significance as an adaptation to xeric conditions. He also showed that the distribution of interxylary cork was systematically important in *Artemisia*, and suggested that it should be included in any future revisions of the genus. The third cambial variant in the Compositae was reported in *Bidens*, *Mikania* (Solereder, 1908; Pfeiffer, 1926) and *Ambrosia* (Jones and Lord, 1982). In these species unequal activity of a vascular cambium produces xylem in restricted areas resulting in distinct lobes. Solereder (1908) suggested that this type of anomalous structure was particularly common in vines, where it provides a high tensile strength and flexibility similar to that of a cable. In *Ambrosia*, Jones and Lord (1982) state that the unequal activity of the cambium eventually results in the splitting of the main axis into several smaller axes, each with their own pith and cambial activity. Jones and Lord (1982) suggested that the production of xylem lobes may have resulted from the earlier initiation of growth each season in areas near axial buds due to the release of hormones from these buds. Jones (1984) found that such "daughter" portions of the plant maintained different xylem pressure potentials which could **potentially** contribute to the differential survival of the portions under stressful conditions. The anomalous cambial activity observed in *Raoulia mammillaris, R. bryoides,* and *R. eximia* in this study, and in *R. mammillaris* by Falvey (1996), is similar to that observed in A*mbrosia*. However, the splitting of the axis and occurrence of bark in the fissures between the xylem lobes, as reported in A*mbrosia* (Jones and Lord, 1982), was not observed in these three species of *Raoulia*. Additionally, the development of lobes in the *Raoulia* species was not as regular as that depicted in A*mbrosia* (Jones and Lord, 1982, their Fig. 13 and 14). It therefore appears that the anomalous cambial activity in *Raoulia* represents a similar, but distinct phenomenon. As such, this characteristic appears to represent an anatomical feature uniting these three species. This grouping, which formed a cluster in the average linkage dendrogram (Figure 2.9), has also been suggested by previous studies, for example *R. bryoides* and *R. mammillaris* were the only mixed species ball cluster formed in the phenetic study of Ward (1993a), while *R. eximia* and *R. bryoides* had a similarity value of one in the phenetic analysis of Breitwieser and Ward (1993) based on their leaf anatomy.

The occurrence of phloem fibres has recently been considered to be an important subtribal character (e.g. Drury and Watson, 1966; Anderberg, 1989; Anderberg, 1991b). The character was first highlighted by Drury and Watson (1966), who showed that the presence of phloem fibres, plus four other anatomical characters, could be used to divide Bentham's (1873) nine subtribes in the Inuleae into two groups. This character was adopted by Anderberg (1989), who included it as part of his descriptions of the tribes Gnaphalieae and Plucheae, for the former noting "stem with phloem concealed with fibres..." and for the latter "generally with distinct phloem". Anderberg also included the presence of phloem fibres in his 1991 study of the Gnaphalieae, stating the presence or absence of phloem fibres appeared to be homogeneous for any one genus, and that all the Gnaphalieae have "a more or less universal presence of phloem fibres". The results of Drury and Watson (1966) record an absence of phloem fibres in *Raoulia glabra* and *Cassina* (*pro parte Ozothamnus leptophyllus*), while Anderberg (1991b) indicates that *R.* subg. *Psychrophyton*, *Cassinia*, and *Ozothamnus* lack phloem fibres. However, these results differ from the findings of this study, as phloem fibres were observed in all species of *Cassinia*, *Ozothamnus* and *Raoulia* studied except *R. hectorii, R. grandiflora* and *R.* sp. "L". Betts (1920a) also recorded phloem fibres in *Ozothamnus leptophyllus*. The difference between the findings of this study and earlier studies may have resulted from the use of material of different stages of development (neither Drury and Watson, nor Anderberg state the age of material they examined) or from different usage of the term "phloem fibres". Drury and Watson (1966) clearly state that their character refers to the presence or absence of fibres **within** the phloem, while Anderberg (1989; 1991b) is less clear, with the wording in the description of the Gnaphalieae implying a bundle cap. It is unclear if either of these studies would have eliminated fibres that may have been interpreted as having developed in the "pericycle". The distinction between pericyclic and phloem fibres has been made in past studies of the Compositae. Esau (1945) stated that the fibre caps in *Helianthus* originated from the primary phloem, while Knobloch (1955) suggests that those in *Cichorium intybus* did not originate from phloem but rather from the pericycle. Foweraker (1917) also classified the fibres he observed in *Raoulia* as pericyclic. However, Carlquist (1957a) suggests that no such distinction between pericyclic and phloem fibres can be made since it is technically difficult to identify a true pericycle in the stem of dicotyledons, especially the Compositae. In this study all sclerenchymatous cells between the vascular cambium and the endodermis (or periderm in mature stems) were categorised as phloem fibres. Despite this uncertainty in interpretation, the results of this study only strengthen the results of Drury and Watson,

reducing the number of species without phloem fibres in their Group I, which is characterised by the presence of fibres, from four to two (i.e *Antennaria dioica* Gaertn. and *Helichyrusm argophyllum* (A.Cunn.) Wakefield are the only species in Group I to lack phloem fibres). The widespread occurrence of phloem fibres in the New Zealand Inuleae, and the reported occurrence in the Australian species *Cremnothamnus thomsonii* (Puttock, 1994), does not support the hypothesis that phloem fibres are primarily associated with annual stems (Carlquist, 1959b). The lack of phloem fibres in two of the five study species of *Gnaphalium*, *G. mackayi* and *G. nitidulum*, supports Drury's (1972) contention that these two species are distinct. The occurrence of phloem fibres as a mass in which individual cells are difficult to detect appears to be characteristic of *Raoulia* subg. *Raoulia,* being present in seven of the species examined, and absent only in *R. cinerea* and *R.* sp. "M". It may also be a general characteristic of the pulvinate species of *Raoulia* subg. *Psychrophyton*, being observed in the three species examined, but investigation of the other three pulvinate species of this subgenus, and the other species in subgenus *Raoulia*, is needed to confirm this. It is notable that the undescribed pulvinate species, *R.* sp. "L", and the four mat species of *Raoulia* subg. *Psychrophyton*, lack these fibres.

The endodermis offers a number of features of potential systematic value, including the presence or absence of a Casparian strip, the general thickening of the endodermis walls, and the endodermis as the primary outer layer of the mature stem.

The occurrence of a Casparian strip is often regarded as the distinguishing feature indicating the presence of an endodermis (e.g. Preistley and North, 1922; Dickison and Weitzman, 1996). Metcalfe (1983d, p. 171) states that for systematic purposes, however, the ease of recognition of a morphologically distinct layer as a boundary between the cortex and the stele is the primary criteria for classifying the cells as endodermal. Although a Casparian strip was present in some species of this study, the distinct morphology of the cells was the criteria used for recording the presence of an endodermis with one notable exception. However, in both *Haastia sinclairii* and *H. pulvinaris* the endodermal cells were morphologically indistinct from the other cortex cells. In these two species the Casparian strip was the only feature indicating the presence of an endodermis. In all other species examined the distinct morphology of the endodermal cells appears to be of potential systematic value. For example, *Pseudognaphalium luteoalbum* and all *Gnaphalium* species, except *G. mackayi*, had radial wall thickenings that were not strongly

birefringent, by comparison all endodermis cell walls were thickened and strongly birefringent in the species of *Cassinia* and *Ozothamnus*. In *Raoulia australis, R. hookeri, R. monroi, R. subsericea, R. glabra, Helichrysum filicaule, H. lanceolatum, Ewartia sinclairii* and *Leucogenes grandiceps* the radial and outer tangential cell walls were thickened and strongly birefringent. Additionally, in these species of *Raoulia*, and possibly *L. grandiceps* and *H. bellidioides*, the endodermis formed the principal outer layer at maturity. In all other species included in this study the outer layer was primarily formed by a periderm or epidermis. Thus the combination of cell morphology and the position of the endodermis in the mature stem was characteristic of these species of *Raoulia* subg. *Raoulia*, and may potentially provide a useful systematic character. However, the remaining species of *Raoulia* subg. *Raoulia* need to be examined to confirm this.

The occurrence of a Casparian strip in the aerial parts of dicotyledonous plants is uncommon (van Fleet, 1961). Metcalfe and Chalk (1983) provide a list of 25 dicotyledon families in which Casparian strips have been observed in the stem. In the Compositae they list the genera *Cichorium, Erigeron, Felicia, Tagetes* and *Xanthium*. In addition Casparian strips have also been observed in *Senecio vulgaris* (Worden, 1935); *Tithonia, Fitchia, Oparanthus, Petrobium* and *Bidens* (Carlquist, 1957a); *Dubautia* (Carlquist, 1959c); *Madia* and *Raillardella* (Carlquist, 1959a); *Zinnia, Chrysanthemum, Ageratum* and *Cosmos* (Wilton and Roberts, 1936). None of these species belong to the Inuleae. Thus the occurrence of a Casparian strip in 13 New Zealand and one Tasmanian species of Inuleae appears to represent the first observation of this feature in the tribe. Given the limited distribution of the Casparian strip in aerial parts of dicotyledonous plants, the presence of a Casparian strip represents a potentially useful systematic indicator. For example, Hufford (1992) suggested that the presence of a Casparian strip in the foliar endodermis of three species of *Synthyris* (Scrophulariaceae) probably represented a derived feature indicating monophyly. He also suggested that the absence of a foliar endodermis in three species of *Besseya* may represent a synapomorphy. In the New Zealand Inuleae the presence of a Casparian strip appears to be a positive indicator of relationship, supporting groupings which have already been identified (Breitwieser and Ward, 1993; Ward, 1993b), for example, the grouping of the three woody whipcord *Helichrysum* species, and the distinctness of *Raoulia cinerea* and *R.* sp. "L".

Closely associated with the endodermis, the resin canals were a prominent feature that separated *Haastia pulvinaris* and *H. sinclairii* from the other species in this study. The presence of secretory canals is thought to be of considerable systematic value (Solereder, 1908, p. 1095; Metcalfe, 1983d) and has been used as a systematic character in several studies in the Compositae (e.g. Carlquist, 1959c; Drury and Watson, 1966; Anderberg, 1989; 1991a; 1991b). While resin canals have been reported in the Inuleae (e.g. Drury and Watson, 1966) they have not been reported in the Gnaphaliinae *sensu* Bentham. The presence of resin canals in the leaf sheath and cortex and the indistinct endodermis morphology of *H. pulvinaris* and *H. sinclairii* do not support the proposal of Merxmüller *et al.* (1977) to include *Haastia* in the Inuleae, but are in agreement with the results from the leaf anatomy (Breitwieser, 1993).

The occurrence of resin canals in *Haastia sinclairii* requires further mention. Breitwieser (1993) found that resin canals were absent in the leaf blade of *H. sinclairii*. This finding was supported by Todd (1996), who stated that resin canals were also absent from the leaf sheaths, except in *H. sinclairii* "Potts" where canals occurred in both the leaf blade and sheath. On the basis of three main characters (the presence of resin canals in the leaf blade and sheath, the branching of the main vein in the involucral bract, and the occurrence of an elongated flowering stem) Todd suggested that *H. sinclairii* "Potts" should be recognised as a distinct species (Todd, 1996, his Appendix 1), despite this taxon clustering in the middle of the other samples of *H. sinclairii* in his phenetic analysis (Todd, 1996, his Fig. 4.16). The absence of resin canals from sheaths reported by Todd was at odds with the results of this study in which distinct resin canals were observed in the leaf sheath of both samples. The specimens examined in this study represented the "normal" (CANU 37796) and the "Potts" (CANU 37682) forms of *H. sinclairii*. For the characters examined in this study these specimens differed in only three features; the epidermal cell shape, the cuticle thickness (both characters which were found to be extremely inconsistent in the stem of all species examined), and the degree of lignification of the pith. The pith in the sample of *H. sinclairii* was completely lignified, while the "Potts" sample still had a few unlignified cells in the centre of the pith. Given the incongruence between the results of this study and that of Todd (1996), the available preparations of the leaf sheath used by Todd were re-examined. Upon re-examination resin canals were apparent in the leaf sheath of all three available samples of *H. sinclairii* and in the leaf blade and sheath of all three available samples of *H. recurva*, which included *H. recurva* var. *wallii* Cockayne. (Todd had

reported that resin canals were also absent from the leaf sheath and blade of *H. recurva.*) Given these findings, the current state of knowledge, and in particular that of the stem and sheath anatomy, do not appear to support Todd's suggestion that *H. sinclairii* "Potts" should be recognised at the species level.

Metcalfe and Chalk (1950, p. xvi) state that the "petiole is of considerable taxonomic importance, since its structure appears to be but little affected by environmental change". They also indicate that in many species the characteristics of the petiole may change as sections are taken from different positions. For this reason the term leaf sheath was used in this thesis, since the observations only represent the nature of the petiole near its base, just above the point at which it attaches to the stem, and not the entire petiole. The inclusion of sheath characters in study of stem anatomy may be justified on the basis that the stem, node and leaf represent a continuum of cells and tissue (Howard, 1983). This continuum is perhaps best exemplified by the relationship between the bundle sheath and the endodermis. The endodermis was observed to extend around the leaf traces as they departed the vascular cylinder. As the leaf traces moved further away from the vascular cylinder endodermal tissue developed between the leaf trace and the leaf gap, thus ensuring a complete endodermal layer surrounding the leaf traces and the vascular cylinder. The tissue of the endodermis and bundle sheath of the leaf and petiole therefore appear to form an unbroken layer around the vascular tissue and may be considered to be homologous. This close relationship between the endodermis and bundle sheath (and the stem-leaf) is further supported by the occurrence of two bundle sheath layers in the four New Zealand species of *Anaphalis*. The double layer in the leaf also occurs in the endodermis of the very young stem, just below the stem apex. However, while the endodermis is reduced to a single discernible layer, the double layer of the bundle sheath in the leaf sheath is maintained. This double bundle sheath layer, which was first reported by Breitwieser (1990), represents a synapomorphy uniting these species in the cladistic analysis, and is one of only three synapomorphies that did not show reversal or parallelism in the analyses.

The presence of sclerenchymatous cells in the sheath mesophyll and epidermis, and as caps on the veins of the leaf blade were reported by Breitwieser (1990). A number of differences occur between the results reported by Breitwieser for the leaf blade, and results obtained here for the leaf sheath. For example, Breitwieser noted the occurrence of sclerenchymatous cells in the bundle sheath of the blade in only *H. lanceolatum*, while in

the leaf sheath sclerified bundle sheath cells were observed in of seven species, including *H. lanceolatum* (One of these species, *Raoulia subulata,* was not included in Breitwieser's study.) Similarly, Breitwieser observed sclerenchyma caps on the leaf blade veins of nine species. Of these nine species sclerenchyma caps were not observed on the leaf sheath veins of two (*Raoulia eximia* and *R. hectorii*), but were recorded in an additional two species, *Ewartia planchonii* and *R. subulata,* and as a complete cylinder in *E. meredithiae*. The affinities suggested by the distribution of sclerenchyma in the leaf sheath include the pairings of *E. meredithiae* and *E. planchonii*, and *Raoulia subulata* and *R. hectorii*, while the occurrence of abaxial sclerenchyma caps in the sheath veins appears to characterise the whipcord species of *Helichrysum* and *H. lanceolatum*.

Another feature noted by Breitwieser (1990) was the occurrence of a prominent space under the abaxial epidermis in the leaf blade of *Raoulia grandiflora*. In this study these spaces were found to extend from the cortex into the sheath in a number of species. These spaces do not appear to be an artefact as no cell damage was observed and the distances along the edges on either side of the spaces were of unequal length. Furthermore, the space appears to be characteristic of some species, with prominent spaces under the epidermis occurring in a number of *Raoulia* and *Helichrysum* species, including *R.* sp. "L", *R.* sp. "M", *R. youngii*, and *R. grandiflora*, and a prominent space separated from the epidermis by a layer of cells occurring in *Haastia pulvinaris*, *H. sinclairii*, and *Ozothamnus leptophyllus*.

Three features which were observed inconsistently (biseriate hairs, cuticle ridges, and crystalline bodies) appear to offer good potential as systematic indicators, if examined by other methods of preparation. The occurrence of different kinds and combinations of trichome are thought to be of considerable taxonomic value (e.g. Solereder, 1908, p 1114), and may delimit species, genera, and sometimes even families (Metcalfe and Chalk, 1950). In the Compositae the study of Carlquist (1958b) is noteworthy since it clearly illustrates the stages of trichome ontogeny and a series of steps in the evolution of trichome structure. Even though transverse stem sections are not the ideal preparation for examining trichome morphology and complement, the observations that were possible suggest that the distribution of biseriate hairs may be of value in delimiting or supporting taxonomic groupings in the New Zealand Inuleae. For example, the occurrence of biseriate hairs with both swollen and unswollen terminal cells was a distinct feature of *Cassinia aculeata* and

C. longifolia, while all other species examined appeared to lack or have only one kind of biseriate hair.

The cuticle characteristics are also of systematic value, particularly the occurrence of ridges and crests (e.g. Solereder, 1908, p. 1071-2). The limited distribution of the three types of ridges that were observed suggests they may be of systematic value in the New Zealand Inuleae (as each type of ridge appeared to occur in only a few genera), particularly if they were examined using a technique such as SEM, which would allow examination of a greater surface area. The presence of crystals may also provide positive reinforcement for taxonomic affinities. However, Solereder (1908) and Metcalfe (1983b) both caution that crystals may be reintroduced into the plants metabolism and that the occurrence of crystals can be very variable in some plant groups. Variation in the occurrence of crystals was a notable feature in the stems of the New Zealand Inuleae, with crystals being observed in one sample only or different types of crystals occurring in each of the samples of a single species. Despite this a more extensive study of crystal type and distribution may provide positive indicators of relationship, as is suggested by the occurrence of silica bodies in two species of *Gnaphalium*, and the prominence of rod- and rhomboidal-shaped crystals in *Cassinia aculeata* and *C. longifolia*.

The use of different techniques to analyse the same data set has become increasingly common (Kim, 1993). Such an approach has been advocated on the assumption that if the different methods of estimating phylogeny agree, this congruence can be taken to indicate support for the reliability of the estimated tree (e.g. Kim, 1993). A multiple method approach may also be advocated on the grounds that "no method is always 'best'" (Duncan *et al.*, 1990), and further that, because each method of analysis varies in its assumptions and associated problems (see below), a multiple method approach may overcome or negate some of these problems.

A multiple method approach can potentially be applied to most data sets since, in order for the analyses to produce meaningful relationships (whether phenetic or phylogenetic), the data set must be based on homology. Practical difficulties with the use of multiple methods of analysis arise, however, when data sets contain taxa with variable character states, multi-state unordered characters, or continuous characters. When the data set contains taxa with variable character states, many ordination techniques and phenetic

clustering techniques are unable to handle such data unless it is re-coded. However, the use of the stepped-coefficient (developed in Appendix 5) allows such data to be analysed using phenetic clustering and PCoA, while maintaining the original weight of the character in the data set. (Similar coefficients have also been developed to handle variable character states in a data set (see Sneath and Sokal, 1973, p. 182-187), however, these are designed for use with frequency data or require the calculation of values for the mean and standard deviation of the samples, and are therefore unsuitable for multi-state unordered characters.) (It must also be noted that PAUP (Swofford, 1993) handles so called "multi-state taxa" as either uncertainty or polymorphism. In the former case PAUP chooses the most parsimonious of the polymorphic states to assign to a taxon in the reconstruct so that the tree length is minimised (Swofford and Beagle, 1993) (i.e. PAUP effectively ignores all but one of the character states in the taxon). When the polymorphism option is employed PAUP assumes the taxon is a heterogeneous group, in which all but one of the states is derived from a monomorphic ancestor (Swofford and Beagle, 1993). However, this option results in the generation of much longer trees, and will not allow the polymorphic state to be inherited from a common ancestor, even when the polymorphic condition is present in both sister groups. Swofford and Beagle (1993) caution that under certain circumstances (e.g. when irreversible or dollo characters are used) the choice between "uncertainty" and "polymorphism" may result in different tree typologies from the analysis of the same data set. In this study variable state taxa were analysed as "polymorphisms", since any variation did not represent uncertainty about the character states (as they could be identified and coded), but rather represented the presence of different character states in the two samples which were compared. The inability to allow the inheritance of polymorphic character states may have contributed to the placement of some of the taxa as individual branches in the polychotomy towards the base of the cladogram (e.g. *R. australis*, *R. subsericea*). These taxa exhibit a number of shared character states, although some of these are polymorphic. If PAUP had been able allow the inheritance of polymorphic states these taxa may have been grouped to form a clade (as some were in Island 1, Figure 2.3), that may have included *R. hookeri*, *R. australis*, *L. grandiceps* (all these taxa share characters 21, 30 and 49, and three taxa share characters 12 and 51). Despite the problems associated with polymorphic characters, their inclusion was shown to be justified in a study by Wiens (1995), in which he demonstrated that polymorphic characters contained significant phylogenetic information.

When a data set contains multi-state unordered characters, these data cannot be analysed using most ordination techniques, unless the character is re-coded into a series of binary characters. The exception to this is PCoA, which can be used to analyse a similarity matrix which was calculated using a similarity co-efficient such as Gower's General Coefficient. (For this reason PCoA was selected over other ordination techniques which have been recommend for use with "cladistic" data, e.g. detrended correspondence analysis (Parnell and Waldren, 1996), and hybrid multi-dimensional scaling (Faith, 1997). Continuous characters currently cannot to be analysed with cladistic techniques, unless the characters are re-coded into discrete multi-state ordered characters (e.g. Baum, 1988; Goldman, 1988). However, this process is controversial (Strait *et al.* 1996), with debate on coding methodology (e.g. Felsenstein, 1988; Farris, 1990), and even as to whether continuous characters are valid cladistic data (e.g. Pimental and Riggins, 1987; Cranston and Humphries, 1988). In this study the four continuous characters which were measured, were not included in the cladistic analysis.

Phenetic techniques are based upon the measurement of overall similarity (Stuessy, 1990). As a consequence the resulting phenograms depict the phenetic relationships of the selected OTUs, rather than their phylogenetic relationships. However, Sneath and Sokal (1973) suggest that "numerical phenetics will in general give monophyletic taxa because we believe that phenetic groups are usually monophyletic". Such interpretation must be based on the assumption of some degree of uniformity in the evolutionary rates in the different clades (Sneath and Sokal, 1973). Colless (1970) suggests phenograms will provide good estimators of cladograms under less stringent conditions. Critics point out, correctly, the problems of such interpretation when the data set contains convergence or unequal rates of evolution along different branches (e.g. Burgmann, 1985; de Queiroz and Good, 1997). Despite these limitations the interpretation of phenograms as estimations of phylogeny persists (e.g. Hopper and Burgmann, 1983; Sokal, 1986; Kim, 1993; Heijerman, 1996). This interpretation, may in part, be based on observation that phenetic techniques have repeatedly been shown to provide accurate estimates of phylogeny (e.g. Kim, 1993; Heijerman, 1996), and "thus serve as useful techniques for inferring cladistic relationships" (Sokal, 1986). However, even if phenetic clustering techniques are shown to be good estimator of phylogeny, such interpretation must be treated with caution as this technique does not allow character state reconstructions to be viewed (as can be done with cladistics),

or reticulation (using non-overlapping techniques), and will produce a hierarchical structure even if none is present in the data set (Sneath and Sokal, 1973; de Queiroz and Good, 1997). (These last two problems also occur in cladistic analyses (e.g. Sneath, 1975; Bremer and Wanntorp, 1979; Hull, 1979).) More importantly, there must be doubt as to interpreting the branching structure of a phenogram as depicting the evolutionary pathway because phenetic clustering has been found to distort the relationships in the similarity matrix, particularly at lower levels of similarity (Sneath and Sokal, 1973), and to produce odd taxon placements. An example of the distortion of relationships in a similarity matrix is apparent in the analysis of the stem anatomy. For the average linkage dendrogram, the cophenetic coefficient values (Figure 2.10), which provide an indication of the amount of distortion, drop rapidly as taxa or groups of taxa are clustered, particularly at the lower levels of similarity, when large clusters are linked. The complete dendrogram has only a 0.66 correlation with the original similarity matrix, so interpreting the evolutionary pathway from these branching patterns would be unsound. Examples of odd taxa placements are also present in the analysis of the stem anatomy data set. For example, *Helichrysum dimorphum* occupies an isolated position in the average linkage dendrogram, with only a weak phenetic relationship indicated with *Haastia*. However, when the similarity coefficients are compared, *H. dimorphum* showed greatest similarity to *H. parvifolium* (0.640), *H. coralloides* (0.639), *Raoulia* sp. "L" (0.638), then *Haastia sinclairii* (0.628). Another example is the isolated position of *E. meredithiae* in the average linkage dendrogram. This species showed greatest similarity to *E. planchonii* (0.783), *R. grandiflora* (0.688), *R.* sp "L" (0.682), *Pterygopappus lawrencei* (0.681) and *Gnaphalium nitidulum* (0.666), all of which belong to Cluster 9 in the dendrogram (Figure 2.9), yet *E. meredithiae* appears to be exclude from this cluster, at least in part, because of its low similarity to *R. youngii* (0.581). Other examples of slight discrepancies in placement of taxa when they are compared to their highest similarity values include *E. sinclairii*, and to a lesser extent, *Helichrysum filicaule*. Thus the distortion of phenetic relationships means that interpreting the placement of such taxa in the dendrogram as indicating phylogenetic relationships would lead to incorrect conclusions, even if equal rates of evolution and no convergence had occurred. In this study the phenograms are interpreted as measures of overall similarity, in which the branching patterns do not depict the phylogeny of the taxa. However, the occurrence of groups in common with the cladogram does suggest that some clusters may represent robust, potentially monophyletic, groups (e.g. *Anaphalis*, and *Raoulia bryoides* and *R. mammillaris*).

Ordination techniques are based on the analysis of covariance (Sorensen and Foottit, 1992), and attempt to maximise the amount of variation that is explained by as few as axes possible. Ordination techniques have two main advantages. First, while phenetics and cladistics require independence of characters (e.g. Mishler, 1994), this is not required by ordination techniques (Sorensen, 1992). And second, ordination techniques are not required to produce a tree-like hierarchy, so that the distortion created by this constraint is not present. This can be seen in the analyses of the stem anatomy, in which the first three axes on the PCoA were able to explain 80% of the variation in the similarity matrix, compared to a cophenetic correlation of only 0.66 in the average linkage dendrogram. However, the depiction of phenetic relationships in more than two dimension means that it is often difficult to identify distinct groups, or to base a classification on such plots (if so desired) (Sokal, 1986). In addition, ordination techniques have been found not to depict close relationships clearly (Sneath and Sokal, 1973). Ordination techniques also exhibit some sensitivity to the characters and taxa which are included (Sokal, 1986; Sorensen and Foottit, 1992), resulting in slight changes when taxa are added or removed. Because of this Sorensen (1992) suggested that the "groups to be analysed be considered to be monophyletic, and that initially all members be used in the analysis." Despite these limitations ordination techniques provide a useful tool for "investigating the general pattern of variation" (Sneath and Sokal, 1973), and have been suggested as a suitable complement to both cladistic (Parnell and Waldren, 1996; Faith, 1997) and phenetic (Sneath and Sokal, 1973) analyses.

Cladistic analysis is based on synapomorphy (Wiley *et al.*, 1991), or the pattern of branching as indicated by character state changes. The aim of cladistics is to reconstruct a hypothetical phylogeny of the selected taxa. In listing the steps involved in cladistic analyses, Stuessy (1990) states that the first step is to make evolutionary assumptions, including the assumption that the study group is monophyletic. Traditionally monophyly has been defined as a group of taxa that share a common ancestor (Stuessy, 1990), however, the term monophyly has been used in cladistics to refer to ALL the descendants of a common ancestor (e.g. Wiley *et al.*, 1991). The importance of monophyly in cladistic analyses is indicated by Coombs *et al.* (1981) in the following statement:

> "Often we are missing a few taxa and/or have mistakenly included a taxon that does not belong in the group, so that the group for the analysis is not truly

> monophyletic (sensu Hennig). So long as there is little homoplasy, extraneous taxa should appear as out-groups in the analysis, and missing taxa should not affect the gross structure of the cladogram ... In large computer analyses of messy data sets, though, monophyly does matter. Removal of a taxon causes the computer to reconstruct the tree completely, often giving a very different result." Coombs *et al.* (1981, p. 365)

This requirement for monophyletic groups potentially creates two problems. First, in large groups it is often impractical to study all taxa thought to form a strict monophyletic group, and second, even when all, or a large number, of the taxa can be included, the analysis of the resultant data set is extremely slow and usually relies upon heuristic search methods. Reliance on heuristic search methods is not desirable, as heuristic methods do not guarantee to find the shortest tree, and may become stuck on local optima (or islands) (Swofford and Beagle, 1993). This was clearly demonstrated in the present study, in which the large number of taxa (51) meant that heuristic search methods had to be employed. The six searches which were conducted appeared to recognise five local optima, each with slightly differing typology (although searches 2, 3, and 5 may have represented different portions of the same island, but because of the maxtrees limit (i.e. the number of trees that could be stored) appeared to be different islands.) Thus, the evolutionary hypothesis generated by a cladistic analysis may depend upon which island(s) is located.

The initial assumption of monophyly also has important implications for the rooting of the cladogram, which is most frequently done by the use of an outgroup. By the inclusion of outgroup taxa in an analysis, the assumption of (strict) monophyly is violated. However by enforcing a monophyly constraint on the analysis, so that only trees consistent with this assumption are kept, the initial assumption of monophyly cannot be tested. In a review of outgroup methodology, Nixon and Carpenter (1993) recommended that cladistic analyses should not be constrained, but rather any outgroup taxa should be treated in the same manner as the ingroup taxa. Once the shortest tree has been found, they suggest that the tree should be rooted between the ingroup and the outgroup. However, if the outgroup taxa are spread amongst the ingroup taxa, the conclusion must be that, on the basis of the data analysed, the ingroup is not monophyletic (Nixon and Carpenter, 1993).

The cladistic analyses of the stem anatomy were not constrained, and were rooted between the two Tasmanian *Cassinia* species and the remaining taxa, so as to provide a consistent

orientation with the analysis of Breitwieser (1990). (Breitwieser (1990), in the only other detailed cladistic analysis of the NZ Inuleae to date, rooted her cladograms using *Cassinia aculeata* and *C. longifolia* on the basis that these species formed their own distinct cluster in her phenetic analysis, and that they were not included in the *Anaphalis-Helichrysum-Gnaphalium* group (the focus of her study) by Merxmüller *et al.* (1977).) Given that the *Cassinia* species were chosen as the outgroup, the position of the *Haastia* clade requires brief mention, since it was suggested earlier in this thesis (in the discussion on resin canals), and by Breitwieser and Ward (1993), that these taxa should not be included in the NZ Inuleae. Their position in the centre of the tree, rather than in a basal position, indicates that the NZ Inuleae is not monophyletic. However, such a conclusion may not be justified, and must be treated with caution for the following reasons. First, not all of the New Zealand or Tasmanian taxa were included in the study, so the initial assumption of (strict) monophyly cannot be made. Second, when *Haastia* is removed, and the analysis re-run, the tree typology remains unaltered (results not presented). And third, the major clades in the cladistic analysis are only supported by 1 or 2 characters which often show reversals higher in the clade, or are parallelled in other clades, this includes the placement of *Haastia* in the clade with four of the woody *Helichrysum* species; a position which is supported by only two characters (the presence of a casparian strip, and a lignified endodermis), both of which are parallelled in other parts of the tree. It is also notable that *Haastia* shows only a weak phenetic relationship to the other NZ taxa on basis of stem anatomy. Its position must therefore be considered an anomaly, possibly resulting from a convergence with other woody alpine species. However, the possible non-monophyly of the NZ Inuleae is also indicated in the cladistic analysis of the leaf anatomy by Breitwieser (1990): in her cladogram *Haastia* is also located in the middle of the tree, rather than in an outgroup position.

Thus, given the limitations of each of the different methods of analysis, it appears that the best approach is to produce trees by several different methods and compare the results. Groups which were in common between in the cladistic and phenetic analyses were *Haastia, Cassinia* and *Ozothamnus*, *Anaphalis*, *Raoulia bryoides* and *R. mammillaris*, *R. glabra* and *R. monroi*, *Ewartia sinclairii* and *Helichrysum lanceolatum*, and *R. petriensis* and *E. planchonii*. While the first four groups have also identified in at least one of the earlier studies (Ward, 1993b; Breitwieser and Ward, 1993), and are therefore supported by other data sets, the last three groups have not been previously identified and

should therefore be treated with caution. Overall, however there is only poor agreement between the different methods of analysis, perhaps reflecting a paucity of systematic information in stem anatomy, or a lack of monophyly, or perhaps indicating complex evolution patterns in the NZ Inuleae, such as reticulation or rapid divergence. All the results must therefore be treated with caution (as hypotheses of phenetic and phylogenetic relationships), particularly as the analyses are only based on data from the stem anatomy. Despite the limitations of the analyses methods, the results provide a means of depicting the relationships in a complex set of data so they can be compared with previous studies (e.g. Ward 1993a, b; Breitwieser and Ward, 1993).

The group formed by the four species of *Anaphalis* is one of only three generic groups that occurs in both the cladistic and phenetic analyses. These species also formed a close group on the basis of their morphology (Ward, 1993b), flavonoids and leaf anatomy (Breitwieser and Ward, 1993). However, the association of *Helichrysum bellidioides* with these species, as suggested by their morphology and flavonoids, was not supported by the stem anatomy, or by the leaf anatomy (Breitwieser and Ward, 1993). On the basis of the stem anatomy *H. bellidioides* appears to have affinities to *Raoulia* subg. *Raoulia* in the phenetic analysis, and to *R. youngii* and *R. petriensis* in the cladistic analysis, although this latter group is also associated with some species of *Raoulia* subg. *Raoulia* in the large terminal polychotomy. The situation of *H. filicaule* is similar to that of *H. bellidioides*. The affinities of *H. filicaule* to *H. bellidioides* and the *Anaphalis* species suggested by their leaf anatomy and flavonoids (Breitwieser and Ward, 1993) is not supported by the stem anatomy. In the cladistic analysis *H. filicaule* appears to have affinities to *Raoulia* subg. *Raoulia*, while its greates similarities in the phenetic analysis are to *H. lanceolatum* and *Ewartia sinclairii*, with more distant links to *Raoulia*.

Haastia pulvinaris and *H. sinclairii* form the second consistent generic grouping. As stated earlier, these species are separated from the other study species by the occurrence of resin canals and an indistinct endodermis morphology. The stem anatomy thus supports the suggestion of Breitwieser and Ward (1993) that not only does *Haastia* not belong in the Gnaphaliinae, but it also does not have close affinities to *Pterygopappus*, as was suggested by Merxmüller *et al.* (1977).

The third consistent generic group is that of the two species of *Cassinia*, to which *Ozothamnus* shows close affinities. The consistently closer association of *O. leptophyllus* to the Tasmania species of *Ozothamnus*, supports the recent transfer of this species from *Cassinia* (Breitwieser and Ward, 1997). However, the differences between *Ozothamnus* and *Cassinia*, as indicated by analysis of the stem anatomy, appear to be slight. The stem anatomy also supports the statement of Breitwieser and Ward (1997) that the woody species of *Helichrysum* are not congeneric with *Ozothamnus*. Both the cladistic and phenetic analyses indicate that there is not a close affinity between Ozothamnus and the woody *Helichrysum* species.

Helichrysum intermedium, H. coralloides, and *H. parvifolium* showed strong affinities in both the cladistic and phenetic analyses. In the cladistic analysis *H. dimorphum* was also included with these species; however, in the phenetic analysis *H. dimorphum* occupied an isolated position, showing only distant similarity to *Haastia*. By contrast *Helichrysum depressum* shows only weak affinities to the three whipcord species in the cladistic analysis, but a stronger similarity to these species in the phenetic analysis. The affinities between these five species were also observed in the flavonoid and leaf data (Breitwieser and Ward, 1993), although Ward (1993b) found *H. depressum* to be somewhat isolated from *H. parvifolium* and *H. intermedium*, exhibiting greater affinity to *Raoulia* subg. *Raoulia*.

The affinities of the remaining species of woody *Helichrysum*, *H. lanceolatum*, were found to be obscure on basis of the leaf anatomy and flavonoid data (Breitwieser and Ward, 1993). In the cladistic and phenetic analysis of this study, *H. lanceolatum* shows greatest affinities to *Ewartia sinclairii*. Given the otherwise isolated position of *E. sinclairii* on the basis of stem anatomy, as well as the leaf anatomy and flavonoids (Breitwieser and Ward, 1993), the relationship between these two taxa may deserve closer investigation. The PCoA also suggests that these two species may have affinities to *Ozothamnus* and *Cassinia*.

The three Tasmanian species of *Ewartia* included in this study do not associate closely in either type of analysis. In both analyses, all three species show as great or equal an affinity to species of *Raoulia* and *Gnaphalium* as they do to each other. Breitwieser and Ward (1993) also found that the *Ewartia* species did not associate closely, and observed a strong

similarity between *Gnaphalium mackayi* and *E. planchonii*. These findings appear to have led Breitwieser and Ward (1993) to suggest that the boundaries between these two genera (i.e. *Ewartia* and *Gnaphalium*) needs revising. The stem anatomy has not clarified this situation, only suggesting closer associations for *E. planchonii* and *E. catipes* to different parts of *Raoulia* and *Gnaphalium*.

The five species of *Gnaphalium* included in this study do not form a monophyletic group, although, in both the cladistic and phenetic analyses, some affinity between these species is apparent. In the cladistic analysis all the species are included in the terminal polychotomy, however *G. audax* and *Pseudognaphalium luteoalbum* are grouped with three species of *Raoulia* and *Helichrysum filicaule*, while the remaining species occur as single branches. In the phenetic analysis *G. audax, G. traversii,* and *G. involucratum* form a cluster with *P. luteoalbum, R. cinerea, Rachelia glaria,* and *Ewartia catipes*. The close association of *Pseudognaphalium* to these species of *Gnaphalium* is not supported by the leaf anatomy, morphology, or flavonoids, each of which found *Pseudognaphalium* to be isolated (Breitwieser and Ward, 1993; Ward, 1993b). In the phenetic analysis *G. nitidulum* is associated with *E. planchonii*, *Raoulia* sp. "L" and *R. grandiflora*, while *G. mackayi* is somewhat isolated, not showing a close affinity to any one species. Thus the analyses of the stem anatomy do not appear to support the suggestion of a close association between *G. mackayi* and *G. nitidulum* (Drury, 1972; Ward, 1993b). However, the cladistic and phenetic analyses appear to have masked the close affinities between the *Gnaphalium* species that are suggested by the characters individually, possibly because the character states do not occur in all taxa: for example, the occurrence of silica bodies in only *Gnaphalium mackayi* and *G. audax*, the casparian strip in *G. mackayi* and *G. traversii*, and the absence of fibres in *G. mackayi* and *G. nitidulum*, all appear to be good indicators of affinity, but only occur in some taxa.

The species of *Raoulia* included in this study do not form a monophyletic group, although, in a similar situation to *Gnaphalium*, the analyses appear to have masked some strong affinities suggested by individual characters, for example, the occurrence of fibres arranged in a mass in most species of *Raoulia*, and the anomalous cambial activity in *R. bryoides, R. mammillaris,* and *R. eximia*. In *Raoulia* subgenus *Raoulia,* only *R. monroi, R. glabra,* and *R. tenuicaulis* consistently associate together in both analyses. In the phenetic analysis *R. hookeri, R. australis,* and *R. subsericea* also cluster closely with these three species,

along with *Leucogenes grandiceps*, but are quite removed from them in the cladistic analysis. Ward (1993b) suggested that these six species of *Raoulia* (plus other species not included in this study) form the core of a genus. However, *R. haastii*, which was also shown to have a high level of similarity to *R. tenuicaulis* and other species of *R.* subg. *Raoulia* (Ward, 1993b), does not associate with these species on the basis of the stem anatomy. In both analyses *R. haastii* groups with *R. mammillaris* and *R. bryoides*. The isolation of *R.* sp. "M", as shown by the leaf anatomy, flavonoids, and morphology (Breitwieser and Ward, 1993; Ward, 1993b), is again repeated on the basis of the stem anatomy, with the somewhat distant links to *Gnaphalium* and *Raoulia* reported by the earlier studies also being identified by the stem anatomy. The last species of *R.* subg. *Raoulia, R. cinerea*, has also been found to be isolated in previous studies (Breitwieser and Ward, 1993; Ward, 1993b). In the numerical analyses this species is closely associated with *Rachelia glaria*. The similarity between *R. glaria* and *Raoulia cinerea* was also noted by Ward *et al.* (1997a), but these two species did not show strong similarity in their leaf anatomy or flavonoids (Breitwieser and Ward, 1993).

The species of *Raoulia* subg. *Psychrophyton* also failed to form a monophyletic group on the basis of the stem anatomy. As mentioned above, *R. mammillaris* and *R. bryoides* formed a group with *R. haastii*, to which *R. eximia* showed affinities, particularly in the numerical analyses. The close relationship between the six pulvinate species of *Raoulia* was observed by Ward (1993a; 1993b) and Breitwieser and Ward (1993), with Ward (1993b) suggesting that these species from the core of a genus. The affinities of the remaining, non-pulvinate, species are less clear. *Raoulia hectorii* and *R. subulata* show affinities to each other, and to *Leucogenes leontopodium* in both the cladistic and phenetic analyses, while *R.* sp. "L" and *R. grandiflora* show weak affinities to each other and to *Gnaphalium* and *Ewartia*, particularly in the numerical analyses. The last species included in *Raoulia* subg. *Psychrophyton, R. youngii*, consistently showed affinities to *Pterygopappus lawrencei* and *Raoulia petriensis* in the phenetic and cladistic analysis. However, the PCoA indicated that the relationship between these three species is not particularly close. Thus not only do these five non-pulvinate species not associate closely with the three pulvinate species, but, also on the basis of their stem anatomy, they do not group closely to each other. This result agrees neither with the findings of Breitwieser and Ward (1993) nor Ward (1993b), which both indicate that, with the exception of *Raoulia grandiflora* (and *R. youngii* in Ward (1993b)), the non-pulvinate species were more similar

to the other species in *R.* subg. *Psychrophyton* than to any other species. The association of two non-pulvinate species and *Leucogenes* was also noted by Ward (1993b). However, the association observed by Ward was between *R. grandiflora*, *R. youngii* and *Leucogenes*, not *R. subulata, R. hectorii* and *Leucogenes* as indicated by the stem anatomy. Thus, while the stem anatomy has confirmed the close relationship of the three pulvinate species, used independently it has not helped to clarify the position of the other members of *Raoulia* subg. *Psychrophyton.*

The two species of *Leucogenes* included in this study did not show strong similarities to each other. As indicated above, *L. leontopodium* exhibited strong affinities to *Raoulia* subg. *Psychrophyton*, while *L. grandiceps* appeared closer to members of *R.* subg. *Raoulia.* These associations are not supported by the general morphology (Ward, 1993b), or the leaf anatomy and flavonoids (Breitwieser and Ward, 1993), all of which found the species of *Leucogenes* to have strong affinities, and to form a generic group.

In conclusion, the stem anatomy provides a number of characters which appear to suggest associations between some species. These characters should be included in future analyses based upon a wider range of evidence. The groupings suggested by the stem anatomy tend to support the same affiliations which have already been identified in previous studies by Ward (1993b; 1993a) and Breitwieser and Ward (1993), particularly in the grouping of the woody *Helichrysum* species, the *Anaphalis* species, and parts of *Raoulia.* However, the stem anatomy does not help to clarify independently the affinities of the isolated taxa, nor to provide characters which give strong indications of generic limits. The stem anatomy, does however suggest different associations which can be tested, and has identified a number of new evidental areas for investigation.

3. Flowering Phenology

3.1 Introduction

Floral characters are particularly important in the taxonomy of the Compositae (e.g. Small, 1918a; 1918b; 1918c; 1918d; Cronquist, 1977). To comprehend fully the systematic significance of many of these features, an understanding of the floral phenology and pollination may be important. For example, an understanding of pollinator mediated selection on the breeding system or floral morphology may aid polarity or homoplasy decisions in phylogenetic studies.

Flowering phenology - the timing of floral events - is an important component in the evolutionary fitness of an individual because of its influence on the reproductive processes such as pollination and seed dispersal (Johnson, 1993). Flowering phenology may influence these reproductive processes through the onset of fruit maturation (e.g. Kelly, 1992), the predation of seed (e.g. Zimmerman, 1980b), the timing of pollinator availability (e.g. Waser, 1979), and pollinator competition (e.g. Gross and Werner, 1983; Waser, 1983). On the other hand, each of these processes may impose selective forces on the flowering phenology. Kochmer and Handel (1986) state that "when the antagonistic selective pressures have become balanced, the species will have reached 'optimal' flowering times."

Phenology patterns may be analysed at several different levels (Newstrom *et al.*, 1994), most commonly the community, population, individual and flower (e.g. Bawa, 1983; Primack, 1985b; Rathcke and Lacey, 1985). The phenological patterns at each successively higher level are the result of patterns at the lower levels; for instance, the flowering duration and intensity of a population is the result of the synchrony and duration of flowering in the individuals which form the population. In some species additional levels may also be added, for example some tropical trees flower asynchronously on different branches, requiring branch level patterns to be analysed (e.g. Bawa, 1983), while species with inflorescences will require another level of analysis between the flower and the individual (e.g. the Compositae capitulum). In this study the main levels of analysis are the association, population, individual, capitulum, and floret. (The term association is

used rather than community in the context of the results of this study as only the members of the Inuleae are examined, rather than all species present in each community.)

Community

At the community and population levels flowering phenology is often correlated with climatic influences. For example, Robertson (1924) showed that the same 22 species flowered nearly three months earlier in Florida than in Illinios due to a milder winter and an early spring in Florida. In temperate regions, the occurrence of spring and autumn frost may limit the flowering season (Rathcke and Lacey, 1985), while in the alpine environment flowering is often restricted by snow-melt patterns and the short growing season (e.g. Spence, 1989; Kudo, 1992). Dry tropical communities may be limited in their flowering time by the distribution of rainfall (Frankie *et al.*, 1974). In contrast to these patterns, some wet tropical communities flower continuously as a result of the combined flowering periods of the species or individuals within each species (Newstrom *et al.*, 1994).

Biotic factors may also influence the flowering patterns of a community; for example the herbs in deciduous woodlands flower before closure of the forest canopy which may reduce the energy available for flowering, or pollinator availability (Heinrich, 1976; Proctor *et al.*, 1996).

A common characteristic of flowering phenologies at the community level is the consistent sequence in which the species initiate flowering (e.g. Heinrich, 1976; Arroyo, 1990; Struck, 1994; Ghazanfar, 1997). This is particularly striking when the order of flowering and the length of flowering in each species results in a consistent staggered flowering pattern with little overlap between each of the species (e.g. Stiles, 1975; Stiles, 1977; Pleasants, 1980). These flowering patterns have been hypothesised to result from selection for the timing of seed release, or competition for pollinators (Primack, 1985b).

Selection on the timing of fruit maturation may result in changes in flowering times since timing of fruit development is often correlated with flowering time (Primack, 1985b). Because the timing of fruit maturation is considered to be under strong selection pressure in some systems (e.g. Snow, 1966; Thompson and Willson, 1979; Stiles, 1980), selection for the timing of fruiting may result in changes in flowering times (Widen, 1991). However, the ability of some species to delay fruit maturation (e.g. *Celtis occidentalis*

(Thompson and Willson, 1979)) would allow the separate adjustment of fruit maturation and flowering times.

Pollinator competition is hypothesised to result in staggered flowering when the fitness of individuals in a species decreases because their flowering times coincide with another species that shares the same pollinators (Mosquin, 1971). When flowering times of sympatric species overlap competition can occur in two ways. First, species may compete directly for pollinator visits (exploitation competition) (Pleasants, 1983). Second, competition may occur when the pollinator moves between the individuals of both species, reducing the effectiveness of each visit (interference competition) (Pleasants, 1983).

Exploitation competition has been demonstrated in only a few studies (e.g. Rathcke, 1983). In one such study, Gross and Werner (1983) demonstrated that early flowering clones of *Solidago graminifolia* set less seed than later flowering clones due to strong competition for pollinator services. They observed that the primary pollinators, honey-bees, only began visiting *S. graminifolia* after the other species in the community finished flowering.

Interference competition may result in a decrease in the fitness of an individual due to interspecific pollen transfer. This may reduce fitness in three ways; first, the male function may be reduced because less pollen reaches conspecific individuals (pollen wastage) (Pleasants, 1983). Second, the female function may be reduced by clogging of the stigma with improper pollen (Pleasants, 1983; Rathcke, 1983). And third, interspecific pollen transfer may also result in the production of hybrids (Rathcke, 1983). The effect of interspecific pollen transfer has been clearly demonstrated in two studies. Campbell (1985) found that seed production in *Stellaria pubera* was reduced due to interference competition with *Claytonia virginica*. This reduction in seed set was the result of the loss of *S. pubera* pollen due to indiscriminate pollinator foraging (Campbell and Motten, 1985). In the second study, Waser (1978a) observed a reduction in the seed set of individuals in *Delphinium nelsonii* when flowering overlapped with *Ipomopsis aggregata*, probably as the result of stigma clogging caused by interspecific pollen transfer by the hummingbird pollinator (Waser, 1978b). Waser (1983) suggested that a similar interaction between *I. aggregata* and *Penstemon barbatus* resulted in the sequential flowering times observed in populations where these species grow sympatrically.

Rathcke (1983) suggested that selection for separate flowering times should be stronger when interspecific pollen transfer results in hybridisation. She suggested that the production of hybrids would not only result in the loss of pollen and of resource in the production the resultant seed, but may also result in competition if the hybrid offspring survives.

Population

Differences in the synchrony of flowering times of individuals have been shown to be influenced by the microclimate in which the individual grows (e.g. Jackson, 1966; Scott, 1966; Webb, 1976), and by the genotype of the individual (e.g. Pors and Werner, 1989; Widen, 1991; Tarasjev, 1997). The genetic control of flowering time is particularly important, since for selective processes to influence flowering time, the control of flowering must be heritable. Primack (1985b) stated that in order for selection to cause changes in flowering times (1) there must be variation in the flowering times of individuals within a population; (2) some of the variation must be genetically based; and (3) selection must act on this variation in flowering times such that variation in the fitness of the individuals is related to the time of flowering.

At the population level the synchrony and duration of flowering in the individuals in the population has been shown to markedly influence the reproductive success of an individual. Augspurger (1981) showed that individuals of *Hybanthus prunifolius* which flowered out of synchrony with the population received lower visitation rates, and suffered higher rates of seed predation, resulting in a lower level of seed production compared to synchronously flowering individuals. In contrast, Zimmerman (1980a; 1980b) showed that highly synchronised flowering may also result in lower seed set due to higher levels of predation and lower visitation rates. Similarly, English-Loeb and Karban (1992) found that clones of *Erigeron glaucus* with a high degree of synchrony that flowered during the peak flowering period of the population suffered greater levels of seed predation, than more poorly synchronised individuals. Rathcke and Lacey (1985) state that such conflicting results suggest a trade-off between pollination and predation, which depends on the densities of pollinators, seed predators and flowers.

Individual

The phenology of an individual is the result of the number, timing, and longevity of the flowers produced by that plant (Primack, 1985b). Primack (1985a) suggested the longevity of individual flowers will influence the effectiveness of the overall floral display, and the amount of geitonogamous pollination that a plant experiences. Geitonogamy, the transfer of self-pollen between flowers on the same plant, is inevitable in outcrossing species that produce a number of flowers at anthesis at the same time (Lloyd and Schoen, 1992), and may be an unavoidable cost of the requirement of large floral displays to attract pollinators (Snow *et al.*, 1996). For example, Wyatt (1981) found that the longevity of individual flowers in *Asclepias tuberosa* results in considerable overlap in the flowering within an inflorescence. Wyatt suggested that this may enhance the attractiveness of the floral display to pollinators, but may also increase the incidence of geitonogamy as pollen is transferred between flowers on the same plant.

The effects of geitonogamy are similar to interspecific pollen transfer, resulting in pollen wastage and stigma clogging (Snow *et al.*, 1996). In self-compatible species, geitonogamous self pollination may also result in the production of offspring which may lack the vigour of outcross offspring (Darwin, 1876), or result in shortened flower longevity due to pollination-induced senescence, resulting in reduced pollen dispersal (Aizen, 1993). The only species which can avoid geitonogamy are those which open a single flower each day, are dioecious, or have strongly synchronised dichogamy across the whole plant (Snow *et al.*, 1996).

Flower

In hermaphroditic species, which comprise approximately 80% of all flowering plants (Proctor *et al.*, 1996), the flowers must function as maternal and paternal parents, both receiving and dispersing pollen. In species pollinated by animal vectors, this requires that the pollen and stigma are presented in approximately the same position so that the same part of the animal will contact pollen and stigmatic surfaces in successive visits (Lloyd and Webb, 1986). However, the close proximity of pollen and stigma within a flower may result in autogamous selfing (i.e. pollen transfer within the same flower) or interference between the male and female functions (Lloyd and Yates, 1982).

Interference and autogamy at the flower level will have same effect as geitonogamy at the level of the plant, resulting in pollen wastage and stigma clogging, and, in self-compatible species, the production of self-pollinated offspring. In self-incompatible species with sporophytic incompatibility systems, the presence of self pollen on the stigma has been demonstrated to decrease the growth of cross pollen (Howlett *et al.*, 1975; Ockendon and Currah, 1977). Ockendon and Currah (1977) also demonstrated that the presence of pollen from another species reduced pollen tube growth by an average of 69%. Thus the presence of self pollen, whether of a geitonogamous or autogamous origin, or of interspecific pollen, may dramatically reduce the fitness of the female function.

The conflict between male and female functions in hermaphroditic flowers has commonly been overcome by separating the two functions temporally (dichogamy) or spatially (herkogamy) (Lloyd and Webb, 1986; Webb and Lloyd, 1986). When separated temporally the female function may occur before (protogyny) or, more commonly, after the male function (protandry). Traditionally herkogamy and dichogamy have been interpreted as "anti-selfing" mechanisms (Barrett *et al.*, 1996), but the occurrence of self-incompatibility and dichogamy or herkogamy in the same species led Lloyd and Yates (1982) to hypothesise that dichogamy and herkogamy may also result from selection to reduce interference between male and female functions. The strength of interference as a selective pressure is indicated by its implication in the evolution of dioecy (Bawa, 1980) and heterostyly (Barrett *et al.*, 1996).

Compositae

In a recent review of pollination in the Compositae, Lane (1996) noted that many of the published studies have been done on commercial species, or consist of anecdotal observations on the identity of floral visitors. Many of the published phenological studies of the Compositae also concentrate upon aspects of commercial species, such as *Helianthus* or *Helipterum*, including the timing of crops (e.g. Sharman *et al.*, 1989a; Sharman *et al.*, 1989b; Chapman *et al.*, 1993), or optimal planting distances (e.g. Patil *et al.*, 1979). Natural populations of Compositae have been studied to investigate community or population patterns (e.g. Hurlbert, 1970; Jones, 1978; Gross and Werner, 1983; Suzuki, 1993), the genetic control of flowering (e.g. Pors and Werner, 1989; Widen, 1991), secondary pollen presentation (e.g. Small, 1915; 1917a; 1917b; Yeo, 1993), the adaptive significance of ray florets (e.g. Ingram and Taylor, 1982; Stuessy *et al.*, 1986), or the

studies have been theoretically based (Leppik, 1960; Burtt, 1961; Burtt, 1977; Leppik, 1977). The studies of Short (1981) and Erhardt (1993) appear to represent the only recent studies of pollination systems in the Inuleae. Studies of Compositae in New Zealand have examined breeding system (Lloyd, 1972), reported the timing of the flowering season (Allan, 1961; Haase, 1986a; 1986b), or listed the range of floral visitors (Thomson, 1926; Heine, 1937; Primack, 1983).

The Compositae are characterised by small flowers (florets) aggregated into a dense head surrounded by a cup of bracts, the whole structure being termed a capitulum. Neff and Simpson (1990) suggest that lack of pollination studies on the Compositae is the result of the small size of the florets and the complex phenological pattern in each head, both of which provide a barrier to studies. The complex phenology of the capitulum is the result of the structure of the capitulum. The capitulum usually contains numerous florets, the number of which varies between species. These florets are often of two types. First, at the periphery of the capitulum ligulate or filiform florets may be present. These florets are often structurally and functionally neuter or female. Second, the central florets, which are often referred to as disc or tubular florets, are usually structurally hermaphroditic. These florets are protandrous, initially presenting pollen at the top of a fused stamen tube, then, once pollen presentation is complete, presenting the style in the same position. Thus, the presence of two types of florets within a single capitulum provides the first level of complexity. The second level of complexity is created by the centripetal development of the capitulum. (Recently bidirectional organogenesis has also been reported in the Compositae (Harris *et al.*, 1991; Harris, 1995)). The pattern of development, with the sequential opening of florets either individually or in groups, results in a varying number of florets at a range of different phenological stages within a single head. The close proximity of florets at different stages of anthesis means that geitonogamy will be unavoidable. Sun and Ganders (1988) found that an average of 43% of seed in the self-compatible species of *Bidens* resulted from self pollination, of which over 50% were the result of geitonogamy. Thus the phenological patterns of the capitulum have important implications for the fitness of an individual. The complexity of phenological patterns in the Compositae may be further complicated by the grouping of capitula into secondary aggregations (Claßen-Bockhoff, 1996).

The success of the Compositae has, in part, been attributed to the structure of the capitulum (Burtt, 1961; Burtt, 1977) for three reasons. First, the structure of the capitulum, with one ovule per floret, provides an efficient system to explore genetic recombinations (Burtt, 1961). Second, while the capitulum is a specialised and complex structure, it functionally has the attributes of a simple flower (Neff and Simpson, 1990) (i.e. freely exposed pollen and styles, easily accessible nectar), which allows a variety of insects to act as pollinators (e.g. Müller, 1883). Third, the capitulum acts as a single pollination unit, allowing many flowers to be pollinated by a single visit (Leppik, 1977).

That the simple functional nature of the capitulum allows a variety of insects to act as pollinators appears to be generally accepted (e.g. Faegri and van der Pijl, 1979; Proctor *et al.*, 1996), although some Compositae have become specialised to particular modes of pollination. For example, *Artemisia vulgaris* and some *Espeletia* species have adapted to wind pollination, producing hanging capitula, more pollen per floret, and pollen with reduced spines (Garnock-Jones, 1986; Berry and Calvo, 1989). Another example is *Pyrrhopappus carolinianus*. This species has adapted to a mutualisitic relationship with a single species of bee (Estes and Thorp, 1975). The capitulum of *P. carolinianus* opens for a few hours every morning, during which time the bee actively forages for pollen, tearing open the stamen tube before the pollen is presented. The foraging behaviour of the bee ensures cross-pollination; however if the pollinator fails the florets achieve self-pollination by bending the style inward to contact pollen on younger florets.

The New Zealand pollinating fauna lacks specialised pollinators such as long-tongued bees and hawkmoths, and includes only 16 species of butterfly (Godley, 1979). Primack (1978) observed a diverse range of floral visitors to species in the Cass-Craigieburn District, but any given element of the insect fauna was unreliable and occurred in low numbers. This generalised insect fauna has been hypothesised to have resulted in the high proportion of small flowers with a simple structure which may be pollinated by a range of insects (Heine, 1937; Lloyd, 1985). Given that most Compositae are reported to be pollinated by a range of insects, the small size of the capitula in most of the New Zealand Inuleae would seem ideally adapted to the characteristics of the New Zealand pollinating fauna, allowing them to be visited by a wide range of insects. However, other than the scattered information contained in papers on the floral visitors (refs above), there are no published studies of pollination or phenology in the New Zealand Inuleae to test this assumption.

The aim of this study was to document the patterns of flowering phenology at the association, population, individual, capitulum, and floret levels of the species of the New Zealand Inuleae which grow in the riverbed, alpine and grassland habitats in the Cass district, and to use the information gained to answer the following questions. Are the phenological patterns of these closely related species the same? If not, how do these patterns relate to any difference in the habitat, breeding system and the types of pollinators observed? How does the phenology of the capitulum and florets influence the level of geitonogamy and interference between male and female functions? Are the phenological differences related to differences in life history patterns of the species?

3.2 Study Sites

3.2.1 Dry Stream

The study site at Dry Stream (Plate 17D) was located upstream of State Highway 7 on the true right side of the stream, for about 200 m (NZMS K34 059712, 780 m a.s.l.). *Raoulia* species were growing on the modified banks provided by the road embankment (8-10° slope, 150° magnetic), and on the old river terraces on either side of the stream (4-5° slope, 290° magnetic). A population of *Helichrysum intermedium* was growing on rocky bluffs approximately 150m from the road (135° magnetic). This population consisted of approximately 35 to 40 plants. A large population of *Helichrysum depressum* was growing on old river terraces immediately up stream from the bluffs, in addition to scattered individuals on more recently disturbed riverbed between the bluffs and the road. The dominant vegetation on the younger terraces and riverbed was *Raoulia australis*, *R. hookeri*, *R. tenuicaulis* and *Epilobium melanocaulon* Hook.f., and a variety of adventive species including *Echium vulgare* L., *Anthoxanthum odoratum* L., and *Agrostis capillaris* L. The older river terraces were dominated by *Discaria toumatou* Raoul, *H. depressum*, *Muehlenbeckia axillaris* (Hook.f.) Walp., and the introduced grasses *Agrostis capillaris* and *Anthoxanthum odoratum*.

3.2.2 Broken River

The study site at Broken River (Plate 18) was located in the valley below Allan's Basin, and up into Allan's Basin itself. The study site extended from the tree line, at *c.* 1200m a.s.l. (NZMS 260 K34 036857), into the alpine scree at *c.* 1610 m a.s.l. (NZMS 260 K34 031865). At the lower part of the study site *Leucogenes grandiceps*, *Raoulia tenuicaulis*, *R. mammillaris*, *R. glabra*, and *Helichrysum bellidioides* all grow in close proximity along the stream bed and on a small bluff system. At the top of the study site six species are present. *R. subulata, Haastia sinclairii* and *H. recurva* grow on the fell-field/scree areas, *R. mammillaris* and *L. grandiceps* grow on bluff systems, while *R. grandiflora* grows in the alpine tussock grassland. At the lower end of the study site deep localised snow was often present until December, while in the Allan's Basin snow lay in late hollows well into January. The main populations of *Raoulia grandiflora* and *Haastia sinclairii* and *H. recurva* that were studied were located on the front of Allan's Basin (NZMS 260 K34 035863, 1400m a.s.l., 35° slope, 115° magnetic). The *Haastia* species were the dominant

scree vegetation, while *R. grandiflora* grew in open patches in an assemblage dominated by *Chionochloa macra* Zotov, *C. pallens* Zotov, and the *Celmisia* species, *C. lyalli* Hook.f., *C. viscosa* Hook.f. and *C. spectablis* Hook.f. The bluff system on which the tagged individuals of *L. grandiceps* and *R. mammillaris* were growing was located approximately 30-50 m above the tree line, at the bottom of the site. (The bluff faces 20° magnetic.) These two species were the dominate vegetation on the bluff systems. The stream bed at the bottom of the site (150° magnetic, 15° slope) was dominated by *Raoulia tenuicaulis Leptinella pyrethrifolia* (Hook.f.) D.Lloyd and C.Webb, *Helichrysum bellidioides* and several *Epilobium* species. *Raoulia subulata* grew in a late snow hollow in Allan's Basin (Plate 17E) (6° slope, 120° magnetic), where it occurred at the border between sparsely vegetated, fine gravel and an low alpine grassland dominated by *Poa colensoi* Hook.f., *Rhytidosperma* sp., *Phyllachne colensoi* (Hook.f.) Bergg., and *Kelleria croizatii* Heads.

3.2.3 Cass

The study site at Cass was located in two areas. The main area was on the Cass Fan (Plate 17A), behind the University of Canterbury field station (NZMS 260 K34 090962, 580-600m a.s.l., 5° slope, 215° magnetic), while the second area was located across State Highway 7, on the Cass Flats (now owned by Grasmere Station) (NZMS 260 K34 075953, 600 m a.s.l.). *Gnaphalium audax*, *Helichrysum filicaule*, *Ozothamnus leptophyllus*, and *Raoulia subsericea* all grow on the fan, while *Raoulia monroi* and *Gnaphalium traversii* are present on the flats. The vegetation on the main fan at Cass is dominated by *Festuca novaezealandiae* (Hack.) Cockayne, *Ozothamnus leptophyllus, Agrostis capillaris*, *Anthoxanthum odoratum*, and in places, *Discaria toumatou*. *G. audax* grew on a small bank (25° slope, 320° magnetic) which was dominated by *Agrostis capillaris*, *Anthoxanthum odoratum*, *Leucopogon fraseri* A.Cunn., and *Coprosma atropurpurea* (Cockayne et Allan) L.Moore, but also had a comparatively high area that was not covered by vegetation.

3.2.4 Cass River

The study site at the Cass River (Plate 17B) was located on the true right bank approximately 1.8 km upstream from the road bridge (NZMS 260 K34 072953, 600 m a.s.l., slope = 1-2°, 360° magnetic). All plants were located on old stable river bed behind

stop banks, however, erosion of the stop banks during 1994 resulted in the loss of some plants. All riverbed species were represented here, except *Raoulia tenuicaulis* which was absent at the start of the 1995/96 season. The vegetation was dominated by the introduced species *Cytisus scoperiaus* (L.) Link, *Ulex europaeus* L., and *Sedum acre* L., which, on the stable river bed, are replacing the native assemblage of *Raoulia* species, *Sclerathanus biflorus* (J.R. et G.Forst) Hook.f. and *Epilobium rostratum* Cheeseman and *E. melanocaulon.*

3.2.5 Broad Stream

The study site at Broad Stream (Plate 17C) was located downstream from the main road bridge (State Highway 7) on the true right side of the stream (NZMS 260 K34 004966, 630 m a.s.l.; slope = 3-4°, 360° magnetic). Plants of *Raoulia glabra*, and *R. australis* were located from just below the bridge to the bottom of the site, approximately 250 m downstream. Populations of *R. tenuicaulis*, *R. hookeri*, *R. haastii* and *Helichrysum depressum* were located on an old river terrace behind a shingle pile in the stream bed. The dominant vegetation on the recent disturbed riverbed were the *Raoulia* species, while on the less recently disturbed areas *Agrostis capillaris, Anthoxanthum odoratum*, *Muehlenbeckia axillaris*, and *Trifolium arvense* L. were also present.

3.3 Methods

Populations of 19 species were observed at five sites in the Cass/Craigieburn district (see section 3.2) over a period of four years (Table 3.1). At some sites, species which were present were not observed because only a few individuals were present (e.g. *Raoulia subsericea* at Broken River), or because of difficulty gaining regular access to the population (e.g. *Helichrysum intermedium* at Broken River). Visits were conducted to each of these sites on a fortnightly basis from approximately August to June each season. On each of these visits, observations of floral visitors, population, individual, and capitulum level phenology data were recorded, and material collected for the floret and capitulum level phenology, seed set and pollen counts.

All graphs were created using routines written in S-Plus for Windows version 3.1.

The nomenclature used is outlined in the methods of the Stem Anatomy (see page 15).

3.3.1 Association and population phenology

At each fortnightly visit it was noted whether any individuals of a species at each site were at anthesis. A species was recorded as being at anthesis if any individuals of that species were observed in flower. Particular care was taken at the beginning and end of anthesis for each species to ensure that any early or late individuals were observed. The area examined for the population phenology was centred on the location of the tagged individuals (see below), but at the start and end of anthesis for each species, plants were checked up to a radius of approximately 500 m away (depending on the topography of the site).

Climate data were obtained from the Geology Department climate station maintained at Chilton Valley, and from the Craigieburn Forest Park climate station (National Institute of Water and Atmospheric Research Ltd.).

River flow data for the years 1965 to 1996 were obtained for the Waimakariri River from the Canterbury Regional Council. To calculate the probability of a flow exceeding two or three times the long term average flow in any week, the flows from all years were ranked separately for each week (1 to 52). The ranked flows for each week were then plotted against the cumulative proportion of flows for that week, and the cumulative proportions

corresponding to two and three times the long term average extrapolated. The probability that a flow exceeds these values was calculated as 1 minus the cumulative proportion of flows.

	Sites				
Species	Dry Stream	Cass	Cass River	Broad Stream	Broken River
Gnaphalium audax	-	1, 2, 3	-	-	-
Gnaphalium traversii	-	1, 2, 3	-	-	-
Haastia recurva/sinclairii	-	-	-	-	1, 2, 3
Helichrysum bellidioides	n/o	-	-	-	1, 2, 3
Helichrysum depressum	1, 2, 3	-	1, 2, 3	2, 3	-
Helichrysum filicaule	-	1, 2, 3	n/o	-	-
Helichrysum intermedium	1, 2, 3	-	-	-	n/o
Leucogenes grandiceps	-	-	-	-	1, 2, 3
Ozothamnus leptophyllus	n/o	1, 2, 3	-	-	n/o
Raoulia australis	1, 2, 3	-	1, 2, 3	2, 3	-
Raoulia glabra	-	-	-	2, 3	1, 2, 3
Raoulia grandiflora	-	-	-	-	1, 2, 3
Raoulia haastii	1, 2, 3	-	1, 2, 3	2, 3	-
Raoulia hookeri	1, 2, 3	-	1, 2, 3	2, 3	-
Raoulia mammillaris	-	-	-	-	1, 2, 3
Raoulia monroi	-	1 ,2 ,3	-	-	-
Raoulia subsericea	-	1, 2, 3	-	-	n/o
Raoulia subulata	-	-	-	-	2,3
Raoulia tenuicaulis	1, 2, 3	-	1, 2	2, 3	1, 2, 3

Table 3.1: The presence/absence of species in the New Zealand Inuleae at the 4 study sites, and the years in which population phenology data were collected (1 = 1993/94, 2 = 1994/95, 3 = 1995/96, n/o = present, but not observed, - = not present).

3.3.2 Individual phenology

At each of the five sites (see section 3.2) individuals of the study species were tagged using aluminium bar or jeweller's tags. At each fortnightly visit during the 1994/95 and 1995/96 seasons every tagged plant was subjectively scored for flowering intensity using the following system:

- no flowers on the plant 0
- few flowers (< 5% of flowers open) 1
- many flowers (5 - 50 %) 2
- an abundance of flowers (> 50 %) 3

Whenever possible the same individuals were followed over both seasons, however occasionally tags or plants were lost due to flooding or stock.

3.3.3 Cluster phenology

The term cluster is used to describe the secondary aggregations of capitula in *Gnaphalium audax*, *Leucogenes grandiceps*, and *Ozothamnus leptophyllus*.

The cluster phenology of these three species was described by examining flowering material in the field with a 20x hand lens. Observations were also checked by examining field collected material under a stereo-microscope in the laboratory (see Capitula Phenology below).

3.3.4 Capitula phenology

The capitula of all species, except *Ozothamnus leptophyllus* and *Helichrysum depressum*, contain two types of floret. The florets to the periphery of the capitulum, are functionally and structurally female. Because of their narrow corolla tube these peripheral florets are referred to as filiform (F) florets. The florets to the centre of the capitulum, referred to as tubular (T) florets, are structurally hermaphroditic, and in most species these florets are also functionally hermaphroditic. In some species, however, the tubular florets are functionally male.

The capitula of *O. leptophyllus* and *H. depressum* contain only tubular florets in the Cass populations, although some populations of the latter species are known to also contain filiform florets (pers. comm. J.M. Ward).

Field Material

Descriptions of the stages in capitula phenology were recorded either directly from material in the field, or from field collected material back in the laboratory. Material for examination in the laboratory was collected directly into plastic containers (without lids) filled with damp sphagnum, and stored in a chilly bin whilst in the vehicle. Once back in the laboratory the material was placed under a growing lamp during the day, and stored in a refrigerator overnight. Material was used for only three days, after which it was discarded, or used for other purposes (e.g. propagation). All field observations were checked in the

laboratory. Material was examined in the field using a 20x hand lens, and in the laboratory using a Wild stereo-dissecting microscope. Photographs of the capitula stages were taken on Velvia Sensia (Fuji) using a Wild Stero-dissecting microscope fitted with a Wild Photographic Unit, or using an Olympus OM-4 fitted with a 90 mm macro lens or bellows unit.

Glasshouse Observations

A collection of plants, grown from cuttings from a variety of locations, was maintained at the University of Canterbury glasshouses (Plate 17F). The plants were grown in plastic pots plunged into a bed of gravel on raised benches. The potting mix used was a combination of peat, sand, and small gravel chip (approximate ratio 2:2:1), and pots were topped with a layer of small chip. Ventilation was provided by under bench fans and vents in the apex of the glasshouse. In addition, during the summer the outside door to the glasshouse was opened to provide extra ventilation. Summer temperatures ranged from 1°C to 37°C. The benches were watered using a continuous drip system, and occasionally individual pots were watered by hand. Insect pests were controlled with occasional dilute applications of Super Shield™. Plants in bud or flower were not sprayed.

Whenever possible capitula observations were conducted on plants grown from the Cass/Craigieburn area. However, when plants from this area did not flower material from other areas was used.

As each species came into bud, up to 10 capitula per plant were tagged by placing coloured markers beside each capitulum. Occasionally leaves below a capitulum were also marked with permanent pen to aid identification. Observations were conducted daily from October 1995 until late February 1996, and over the same period in 1996/97. At each observation the following information was recorded for each tagged capitulum until it had completed anthesis; the number of filiform florets open, the number of tubular florets presenting pollen, and the number of tubular florets presenting styles.

3.3.5 Floret phenology

Material collected on fortnightly visits was used to describe the patterns of floret phenology. Capitula were examined under a stereo-dissecting microscope, before they were dissected and the florets drawn with the aid of a drawing tube.

3.3.6 Breeding system

Pollen counts

The total number of pollen grains were counted for six tubular florets of each species, except *Raoulia subsericea* for which only four florets were available due to high levels of predation. The florets for each species were taken from samples that were collected, and placed directly into FAA in the field. The six florets were obtained by taking two florets, one from the outside of the capitulum, the other from towards the middle, from one capitulum from each of three different individuals. Once dissected from the capitulum, the florets were placed in distilled water for one to two hours. The florets were then placed in a small drop of water on a clean microscope slide and dissected under a stereo-microscope to open the corolla and anther sacs, and disperse the pollen. A small quantity of aniline blue was then introduced, and the preparation covered with a coverslip.

The slides used for pollen counts had a 1 mm by 4 mm grid printed on thin plastic glued to the underside of the slide. This allowed accurate search patterns to be maintained, and avoided counting pollen grains twice. All pollen grains under the coverslip were counted. No burst pollen grains were observed.

Seed set

The seed set for each species was estimated by counting the total number of filiform and tubular florets in a capitulum, and the number of each type of floret in which the achene was filled. A filled achene was defined as one in which the surface was smooth and rigid, and not transparent to bottom mounted light. The seed counts were conducted on preserved, field collected material. Whenever possible, 30 capitula from at least five different individuals were counted. However, because of high levels of predation in some species (e.g. *Raoulia subsericea*) 30 capitula were not always available, despite more than 60 capitula being collected for each species.

Gender estimates

The female fitness of an individual, G_i, was defined by Lloyd (1980) as

$$Gi = \frac{g_i}{g_i + a_i E}$$

where $E = \frac{\sum g_i}{\sum a_i}$ for all individuals of a species, and g_i is the number of gynoecial units and a_i is the number of androecial units. For gender comparisons within a species the gynoecial units were equal to the number of florets or the number of seed produced, for the phenotypic and functional gender respectively. Androecial units were the number of tubular florets for both the phenotypic and functional gender estimates.

In gender calculation for comparison between species, a_i was equal to the average number of tubular florets multiplied by the average number of pollen grains per floret in that species. The gynoecial units used were the average number of florets and seed set by each species, for the functional and phenotypic gender respectively.

Pollinator Observations

The aim of the pollinator observations was to identify the types of insects visiting each plant species. Observations of floral visitors were conducted during the fortnightly visits to the study sites. The identities of floral visitors to each species were recorded whenever they were observed while in the field. In addition, as each plant species reached the peak in its flowering season, observations of pollinator activity were conducted by watching a few individuals of the chosen plant species for an hour or more. If pollinators were scarce observation periods were longer. For each species the activity of the floral visitors on each species was observed for over five hours. When possible insects were collected for identification when observed for the first time. Most of these insects were identified using the insect collection in the Zoology Department, University of Canterbury. The remaining insects were identified by Professor M. Winterbourn of the same department.

Night observations of *Gnaphalium audax, Helichrysum filicaule, H. intermedium*, and *Raoulia subsericea* were also conducted. For *H. intermedium* these observations were conducted in one three hour period on 8 December 1996. Observations for the other three

species were conducted in four 1-2 hour periods in December 1996, and January and February 1997.

3.4 Results

3.4.1 Association and population phenology

The flowering season for all species of Inuleae observed in the Cass-Craigieburn district extended for 8-9 months, beginning in early September and extending through into April or May of the following year (Figure 3.1). The length of this flowering season results from the staggering of the start dates of the species at each site. This staggering of species occurs in the same order, between both years and sites, with only small fluctuations in some species. A slight staggering of flowering seasons is also apparent between the sites which have species in common. The flowering season of any species begins first in the population at Broad Stream. In contrast, the populations at Dry Stream are consistently last to flower (excluding those at Broken River), while the Cass populations usually begin flowering at a time intermediate to Broad Stream and Dry Stream, although on occasion the populations at Cass were observed to start anthesis at the same time as the Broad Stream or Dry Stream populations. For example, *Helichrysum depressum* started flowering sequentially at Broad Stream, Cass, then Dry Stream in the 1994/95 season, but equally at Cass and Broad Stream in the 1995/96 season (Figure 3.1). *Raoulia hookeri* began flowering at Broad Stream approximately two weeks earlier than the Cass and Dry Stream populations in 1994/95, while in the 1995/96 season the populations are first recorded as being at anthesis on three sequential fortnightly visits at Broad Stream, Cass, and Dry Stream.

A delay in the start time is also apparent in the populations of two species, *Raoulia glabra* and *R. tenuicaulis,* observed at Broken River and at least one other site. *Raoulia glabra,* which was present at Broad Stream and Broken River, flowered four to six weeks later at Broken River than at Broad Stream. The population of *R. tenuicaulis* at Broken River flowered approximately four weeks later than the populations at Cass, Broad Stream and Dry Stream (Figure 3.1).

Further trends become apparent when the population phenologies are examined in terms of three broad species associations: alpine, grassland, and riverbed.

The alpine association was represented by eight species growing at Broken River (Table 3.1), and *Helichrysum intermedium* at Dry Stream (Figure 3.2). (No data are available for *Raoulia subulata* over the 1993/94 season, as this species was not discovered at the site until early 1994. Data were not collected for *Haastia recurva* or *H. sinclairii* in the 1993/94 season.) The most striking feature of the alpine species is the compact flowering season. When *R. tenuicaulis* and *R. glabra* are excluded (see below), the flowering season extends for only four months, beginning in early December and ending in late March/early April. The compact flowering season results from the closely spaced starting dates for anthesis, with only four to six weeks separating the dates of first observed flowering of the first and last for six of the nine species (Figure 3.2). At Broken River *Raoulia tenuicaulis* grows over a wide altitudinal gradient, being present on the stream bed from below the tree line (at *c.* 1040 m) to *c.* 1300 m. A strong altitudinal gradient was apparent in the starting dates across this population. The first flowers at anthesis were recorded in October/ November at the bottom of the population, while the plants adjacent to the tagged populations of *R. mammillaris*, *H. bellidioides*, and *L. grandiceps* did not begin flowering for another 3-4 weeks. Thus at the top of the study site *R. tenuicaulis* only began flowering approximately 2-3 weeks before the other species.

Raoulia glabra occurred at Broken River in open patches at the treeline that quickly became free of snow, and was therefore not truly alpine. This species was not observed to begin flowering until March, by which time the populations of most other species had finished flowering.

Of the remaining alpine species, the flowering pattern of the *Raoulia subulata* population is distinct. In the 1994/95 season the population was only recorded at anthesis on one visit. By comparison in the 1995/96 season the population was observed at anthesis on three consecutive visits. The longer flowering season in 1995/96 appears to correspond to an early snow melt in the hollow where the population was located during that season.

The grassland association was represented by seven species (Table 3.1) observed on the Cass flats and fan (Figure 3.3). The flowering season of the grassland species was observed to last for six to seven months, begining in late October/early November and extending through to April or May of the following year. The flowering season of the

grassland species was more staggered than that of the alpine species, with a period of 10-12 weeks between the start of flowering in the first species, and the start of flowering in the last species. The length of the flowering season for each species population in the grassland assemblage (Figure 3.3) were more variable than those in the alpine assemblage (Figure 3.2), both between years and species. For example, *Gnaphalium traversii* was observed to have the shortest individual flowering season of the grassland species. This species flowered for just four weeks in the 1994/95 season, while in the 1993/94 and 1995/96 seasons the population of this species flowered for 10 weeks. By contrast, the flowering season of *Raoulia monroi* was comparatively constant, flowering over a six to eight week period, giving this species, on average, the shortest flowering season of the grassland species. The five other grassland species had flowering seasons of between 8 to 22 weeks, with the flowering season of *G. audax* being the longest observed in any assemblage. The population of *G. audax* flowered for 16, 22, and 20 weeks in the 1993/94, 1994/94, and 1995/96 seasons respectively (Figure 3.3).

The riverbed assemblage was represented by five species observed at Broad Stream, Cass River and Dry Stream (Figure 3.4). The flowering season of the riverbed species started in late September/early October and continued through until late March or early May, depending on the site and season (Figure 3.4). The combined flowering season for these species therefore extended for just under nine months. The dates of first observed flowering for each species were extremely staggered, with a period of 16 to 20 weeks between the first flowering date in the first and last species to reach anthesis (Figure 3.4). This staggering is made more apparent by the generally short flowering season of the riverbed species, especially *R. tenuicaulis, R. haastii,* and *R. australis*. The populations of these three species flowered for 8-12, 4-8, and 6-12 weeks respectively, while the two later flowering species, *R. hookeri* and *Helichrysum depressum,* flowered for 5-18 weeks and 6-12 weeks, respectively. A prominent feature in the flowering pattern of the riverbed populations is the gap of two to four weeks between the end of flowering in *R. haastii* and the beginning of flowering in *R. australis* (Figure 3.4). This gap was observed in all site-year combinations, and was also observed at the Cass River during the 1996/97 season with a three week gap between the end of anthesis in *R. haastii* and the beginning in *R. australis*. (Dry Stream and Broad Stream were not visited during the 1996/97 season.)

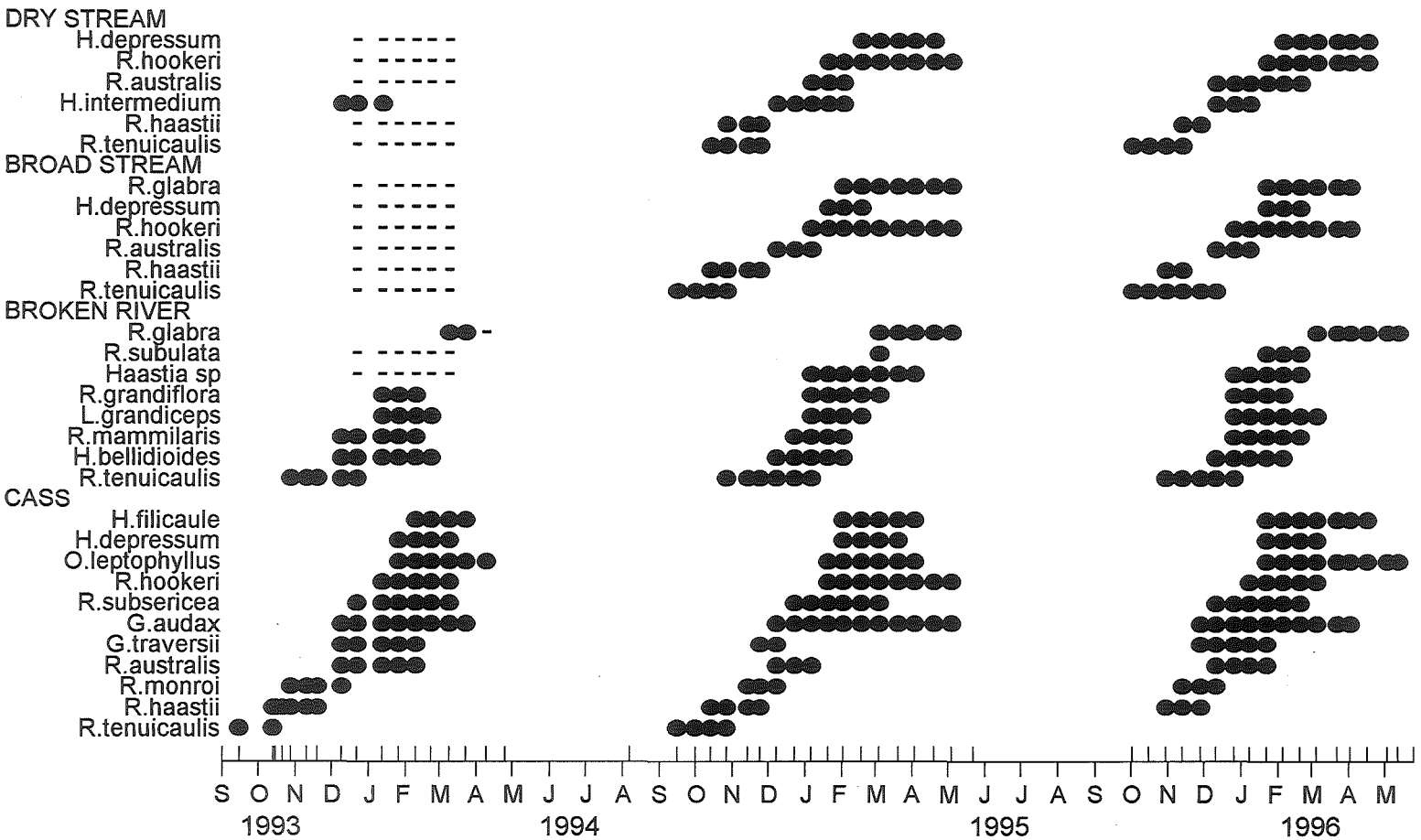


Figure 3.1: The flowering phenology of all species of New Zealand Inuleae examined in the Cass-Craigieburn district during the 1993/94, 1994/95 and 1995/96 seasons. Upper tick marks indicate sampling dates. Circles indicate a population at anthesis. - indicates unknown.

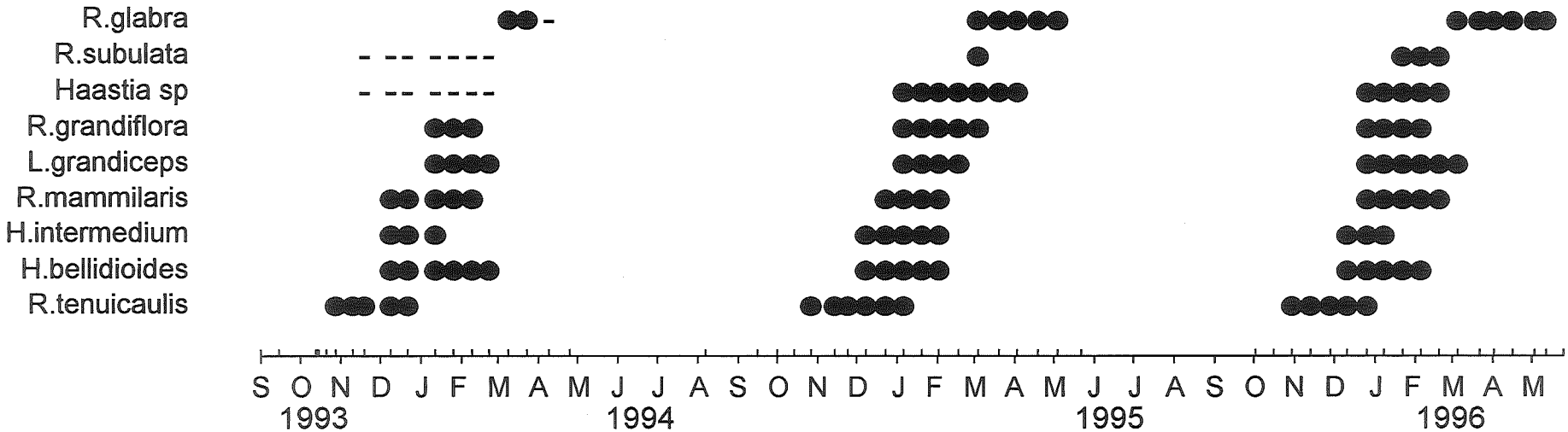


Figure 3.2: The flowering phenology of the populations of alpine species at Broken River during the 1993/94, 1994/95, and 1995/96 seasons. (NB: *Helichrysum intermedium* was monitored at Dry Stream.) Upper tick marks indicate sampling dates.

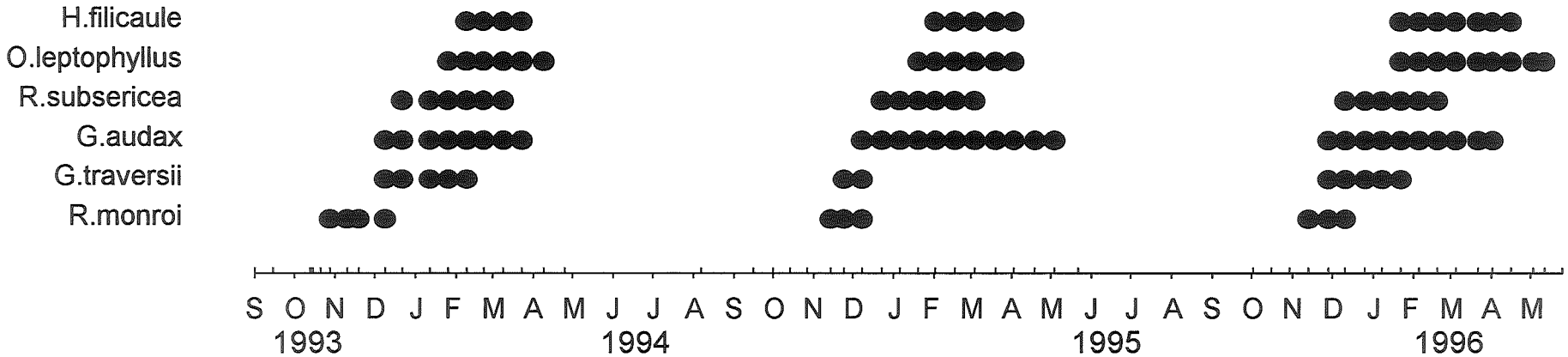


Figure 3.3: The flowering phenology of the populations of the grassland species at Cass over the 1993/94, 1994/95, and 1995/96 seasons. Upper tick marks indicate sampling dates.

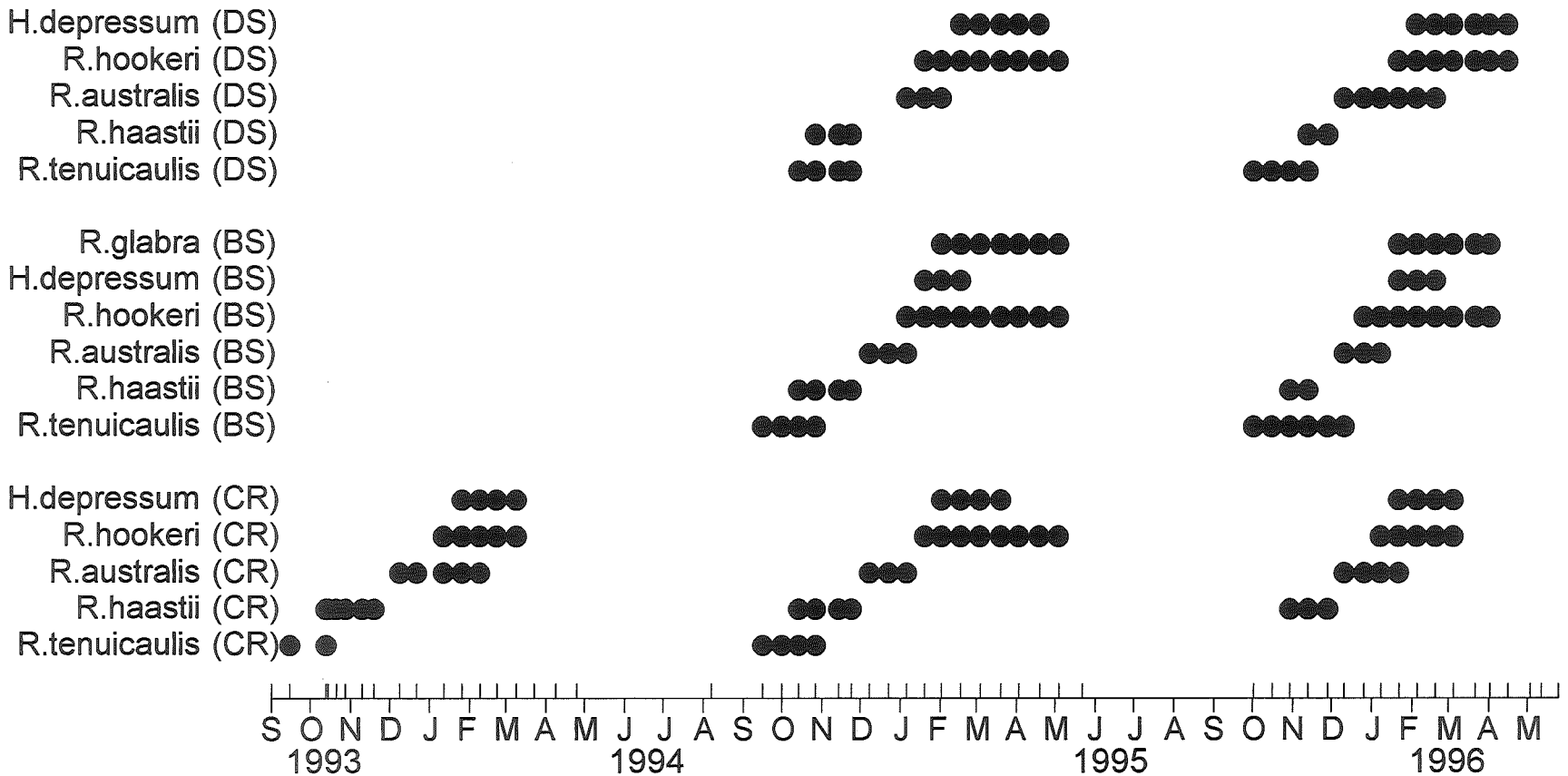


Figure 3.4: The flowering phenology of populations of the riverbed species at the Dry Stream (DS), Broad Stream (BS) and Cass river (CR),during the 1993/94, 1994/95 and 1995/96 seasons. (Broad and Dry Stream not sampled during 1993/94 season.) Upper tick marks indicate sampling dates.

Climate Data

The probability of the water flow in the Waimakariri River exceeding the long-term average by two or three times, gradually increased from August and peaks during November (Figure 3.5). Following the November peak, the probability of high flows gradually decreased, with very little chance of high flows occurring after the end of February.

The rainfall patterns of the 1993/94 and 1994/95 seasons showed markedly different patterns. Greater rainfall occurred in 1993/94 until December, while greater rainfall occurred from January to March of the 1994/95 season. This pattern is apparent despite missing values occurring in some months, since the majority of missing values occurred in high rainfall months (Figure 3.6).

The number of days with average surface temperatures below 0°C during the 1993/94 and 1994/95 seasons showed the same general pattern, with no days below 0°C recorded from October to April (Figure 3.7). In the 1993/94 season this period extended to include May.

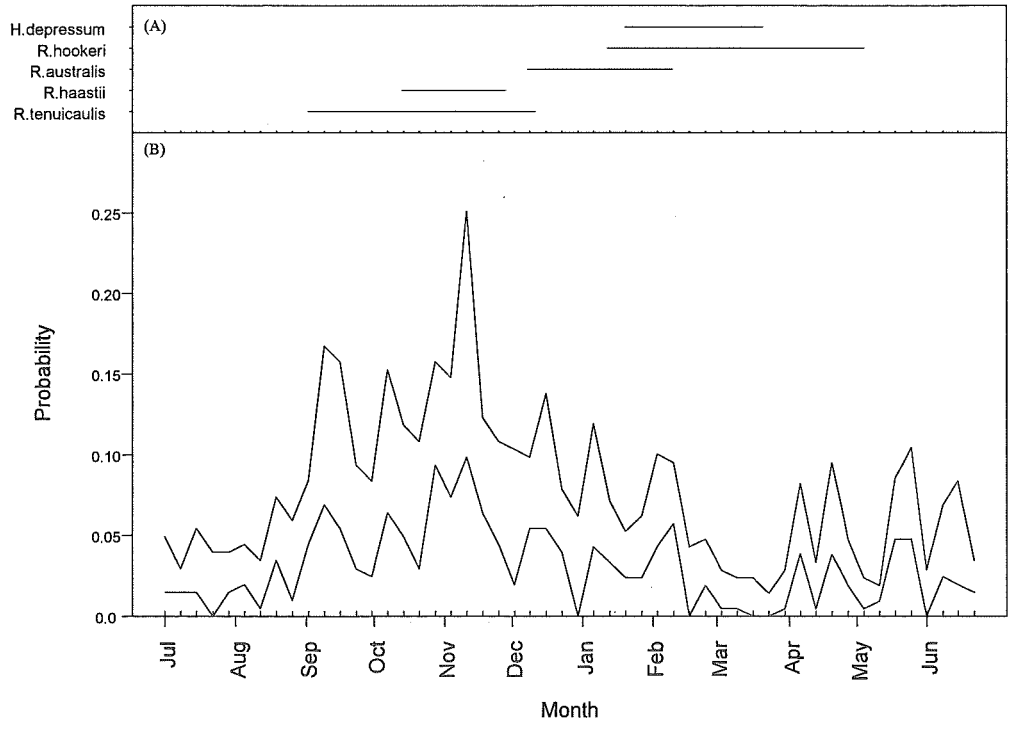


Figure 3.5: The probability of the Waimakariri river flow exceeding the long term average (B) by two (top line) or three times (bottom line), with the maximum length of the flowering season of the five riverbed species as observed at Broad Stream, the Cass River, and Dry Stream over the combined 1993 to 1996 seasons plotted above (A).

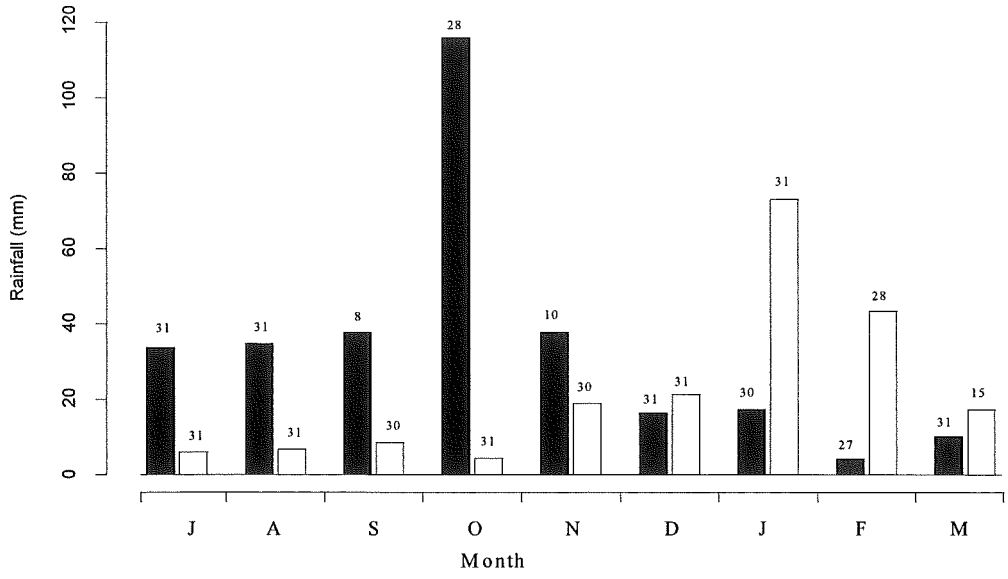


Figure 3.6: The total monthly rainfall recorded at the Chilton Valley over the 1993/94 (solid bars) and 1994/95 (empty bars) seasons. Number above each bar indicates number of daily records available.

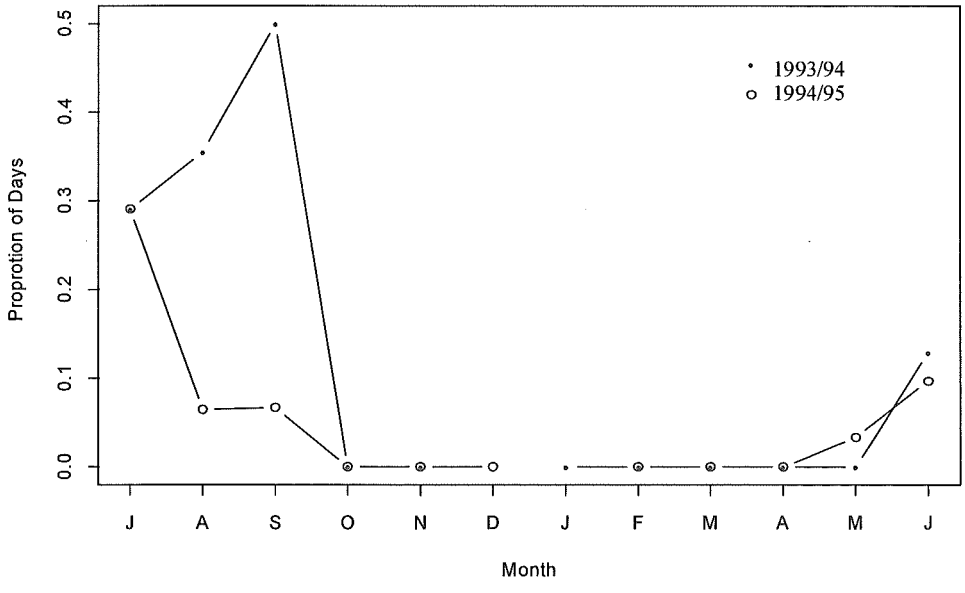


Figure 3.7: The proportion of days sampled in the 1993/94 and 1994/95 seasons in which the average surface temperature recorded at Chilton Valley was less than zero.

3.4.2 Individual phenology

The flowering patterns of tagged individuals for 16 species are presented for each species site combination over the 1994/95 and 1995/96 seasons (Figure 3.8 to Figure 3.29). The

three different dot sizes indicate low (smallest dot), medium and high (largest dot) flowering intensities. For a number of individuals it was not possible to follow them over two seasons, the missing values for these plants are indicated by a "-" sign.

A prominent feature of *Gnaphalium traversii* (Figure 3.9) and *Helichrysum filicaule* (Figure 3.13), and to a lesser extent *Raoulia grandiflora* (Figure 3.22), *H. intermedium* (Figure 3.14), and *H. depressum* (Figure 3.11), is the number of individuals which did not flower in one or both seasons. This contrasted with the other species in which nearly all individuals flowered in both seasons. The degree of synchrony between individuals also varies, both between years and between individuals. For example, the individuals of *R. subsericea* (Figure 3.29) and *R. monroi* (Figure 3.27) showed poor synchrony in their starting dates. In a number of species (e.g. *Ozothamnus leptophyllus* (Figure 3.16) and *H. intermedium* (Figure 3.14)) the individuals appeared to start flowering in approximately the same order each year, with the same individuals usually flowering later each season. In contrast to both the former patterns, the individuals of *R. australis* (Figure 3.17, Figure 3.18), *R. haastii* (Figure 3.20, Figure 3.21), and *H. depressum* (Figure 3.11, Figure 3.12) showed a high level of synchrony in the starting and, particularly in the first two species, the finishing dates of anthesis.

Another prominent feature in some species was the occurrence of non-flowering gaps, or pulses, during the flowering season of some individuals. Such pulses were observed most prominently in *Raoulia hookeri* (Figure 3.23 to Figure 3.25) and *Gnaphalium audax* (Figure 3.8), but were also observed in *G. traversii* (Figure 3.9), *R. subsericea* (Figure 3.29), *R. tenuicaulis* (Figure 3.26), *H. depressum* (Figure 3.11 and Figure 3.12), *H. filicaule* (Figure 3.13) and *Ozothamnus leptophyllus* (Figure 3.16).

The final prominent feature of the individual flowering patterns was the pattern of each individual's flowering intentsity. Most individuals did not usually have the same pattern of flowering intensity in both seasons. Similarly, the individuals of a species were not generally observed to have the same intensity pattern, even within the same year, with the exclusion of *Raoulia australis* and *R. tenuicaulis*. In both of these species, the individuals were observed to have a high degree of similarity between individuals, seasons, and sites (Figure 3.18 and Figure 3.19, Figure 3.25 and Figure 3.26).

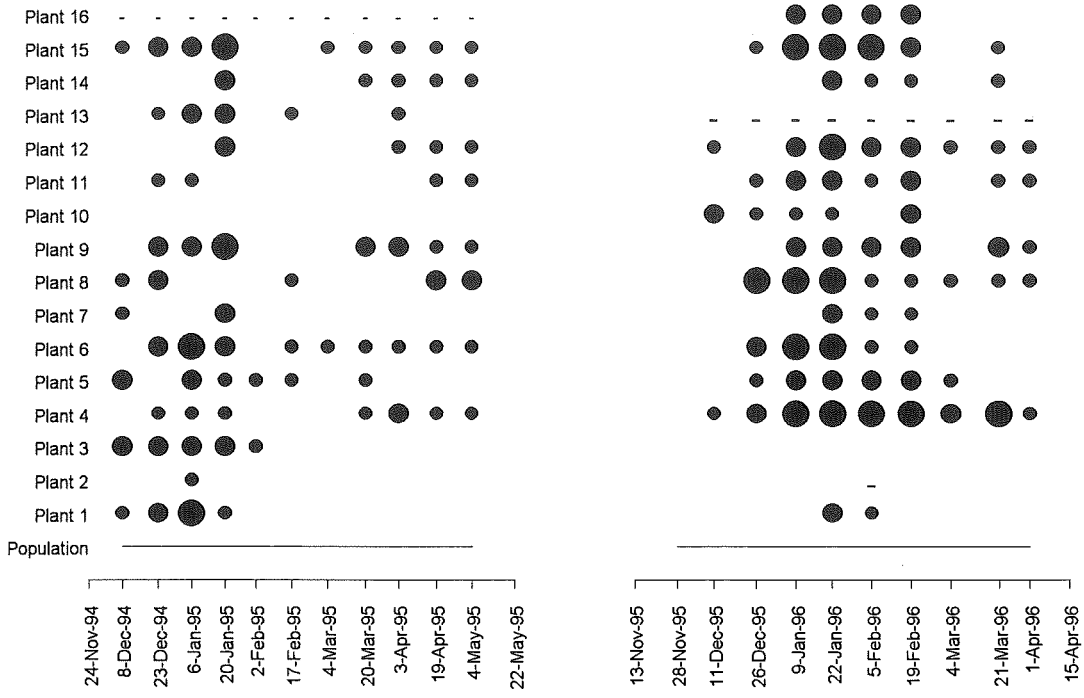


Figure 3.8: The flowering phenology of 16 tagged individuals of *Gnaphalium audax* at Cass over the 1994/95 and 1995/96 seasons. Dots indicate low (small dots), medium and high (large dots) flowering intensity. Blank spaces represent non flowering. - indicate missing data.

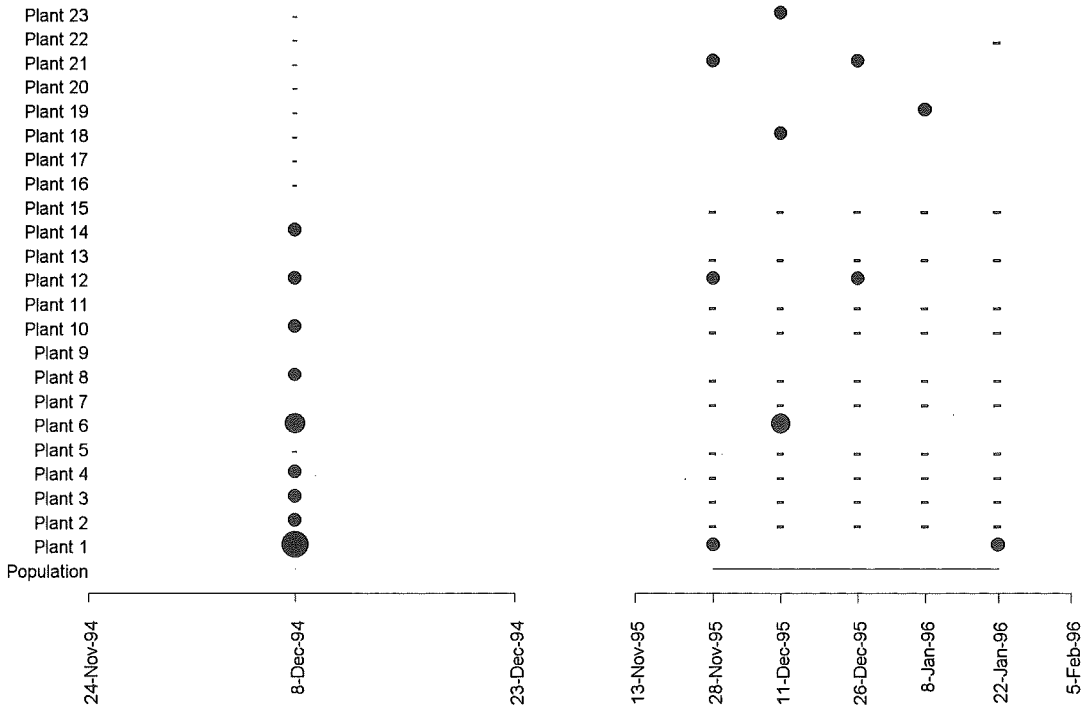


Figure 3.9: The flowering phenology of 23 tagged individuals of *Gnaphalium traversii* at Cass over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

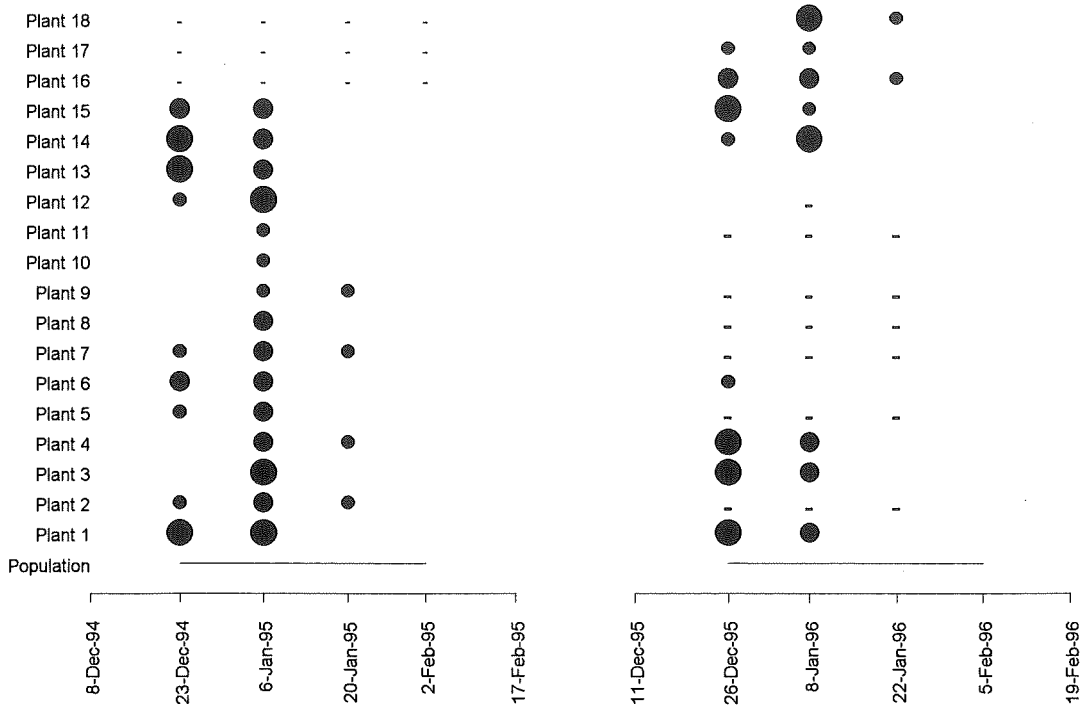


Figure 3.10: The flowering phenology of 18 tagged individuals of *Helichrysum bellidioides* at Broken River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

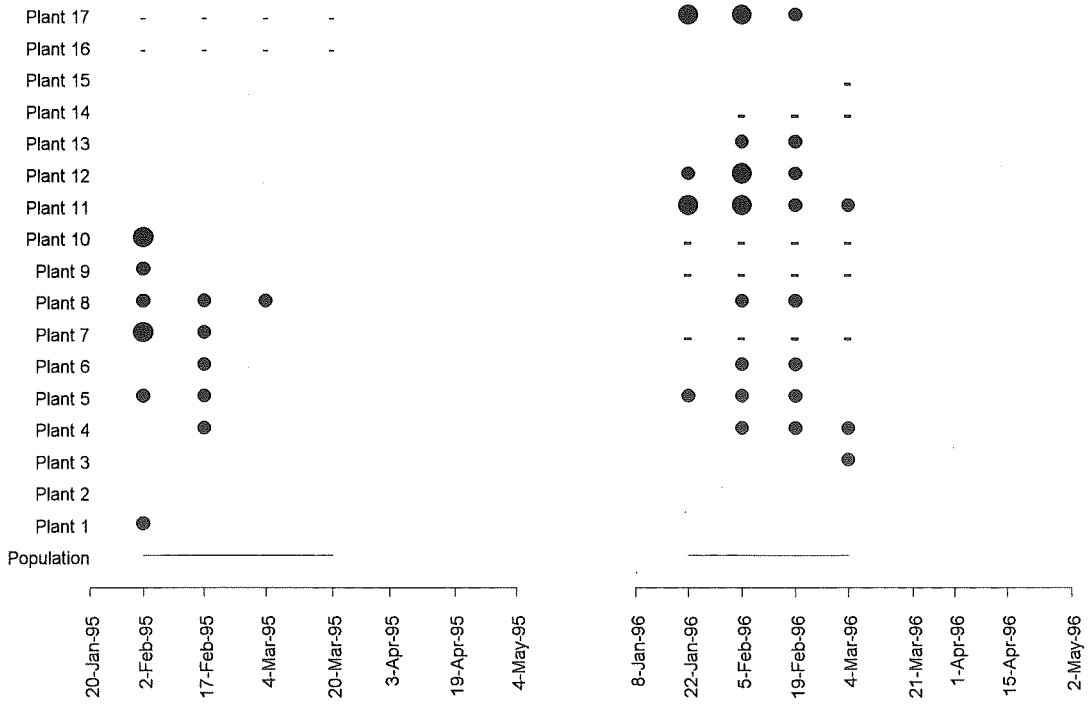


Figure 3.11: The flowering phenology of 17 tagged individuals of *Helichrysum depressum* at the Cass River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

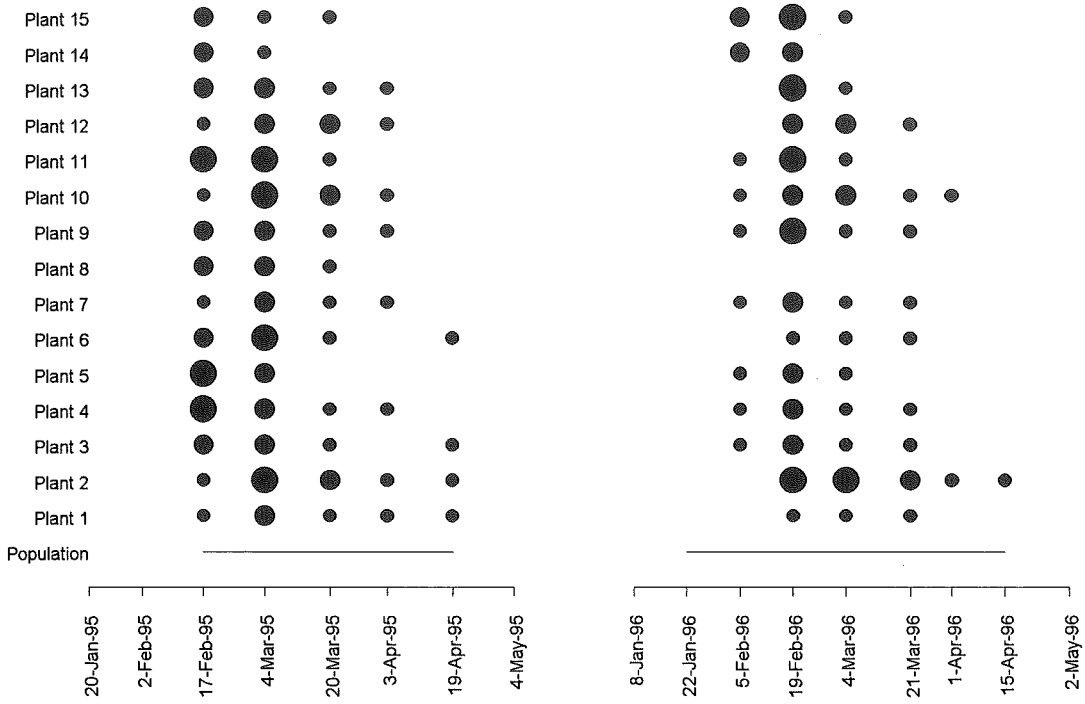


Figure 3.12: The flowering phenology of 15 tagged individuals of *Helichrysum depressum* at Dry Stream over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

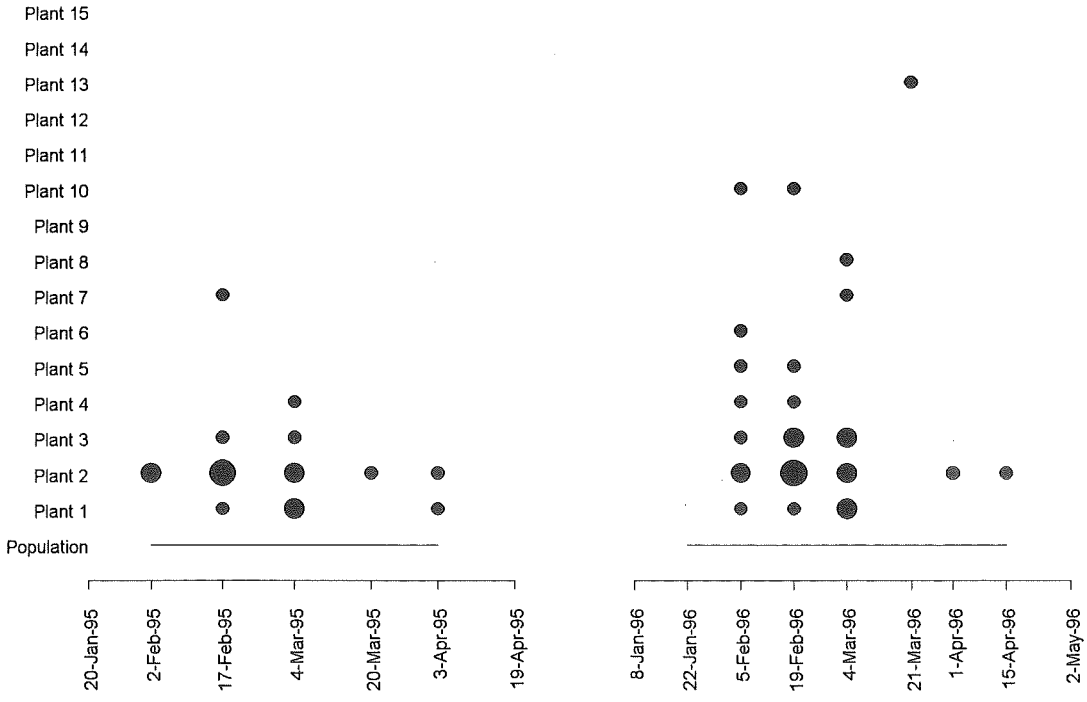


Figure 3.13: The flowering phenology of 15 tagged individuals of *Helichrysum filicaule* at Cass over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

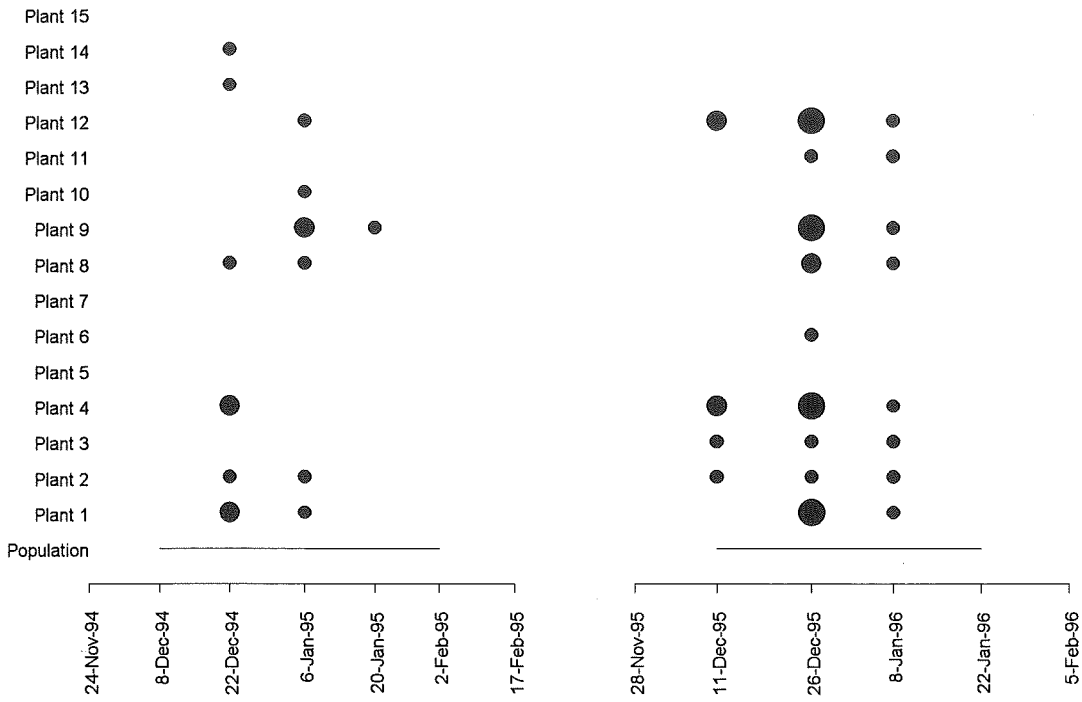


Figure 3.14: The flowering phenology of 15 tagged individuals of *Helichrysum intermedium* at Dry Stream over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

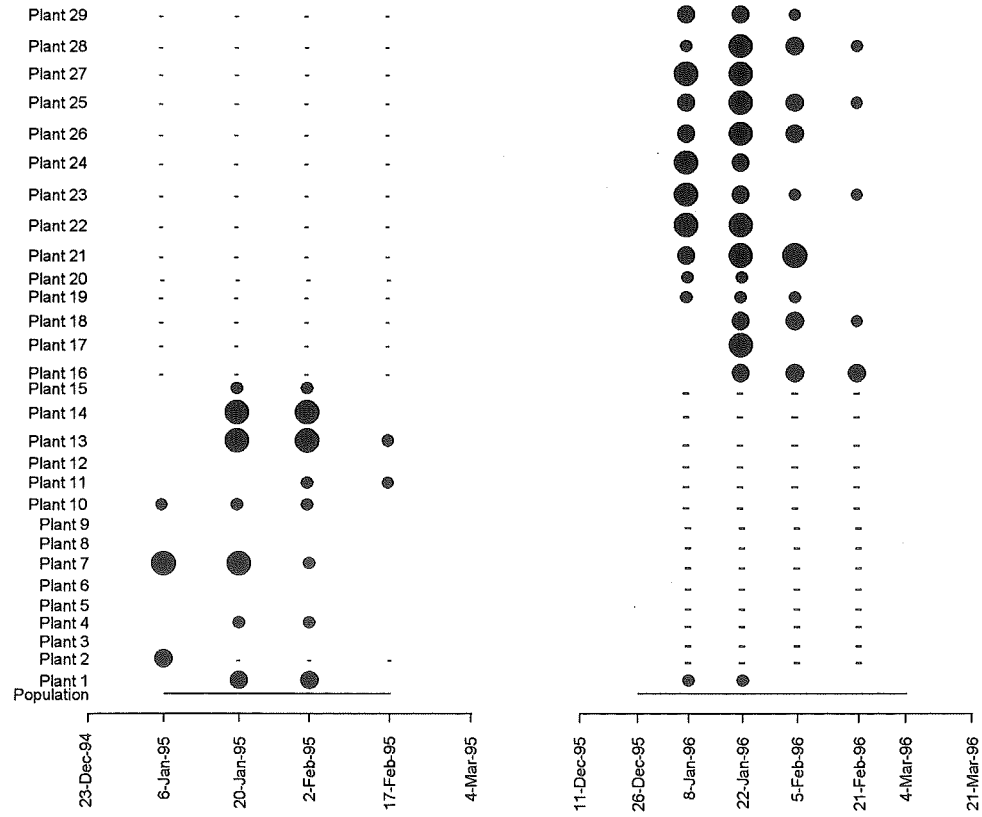


Figure 3.15: The flowering phenology of 29 tagged individuals of *Leucogenes grandiceps* at Broken River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

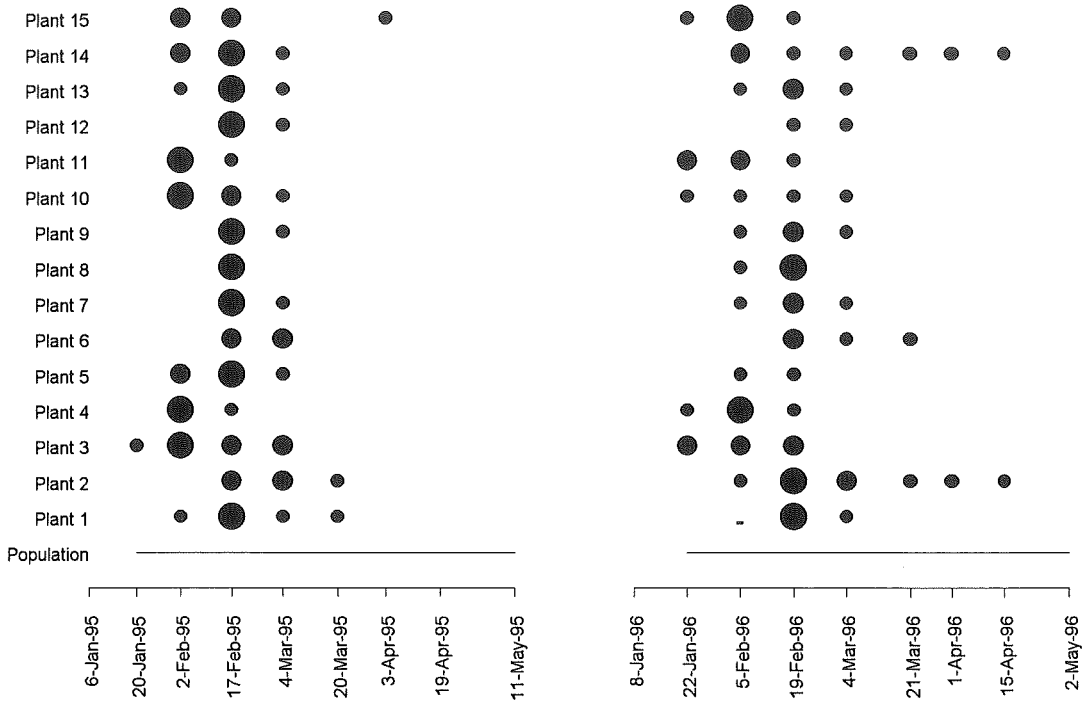


Figure 3.16: The flowering phenology of 15 tagged individuals of *Ozothamnus leptophyllus* at Cass over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

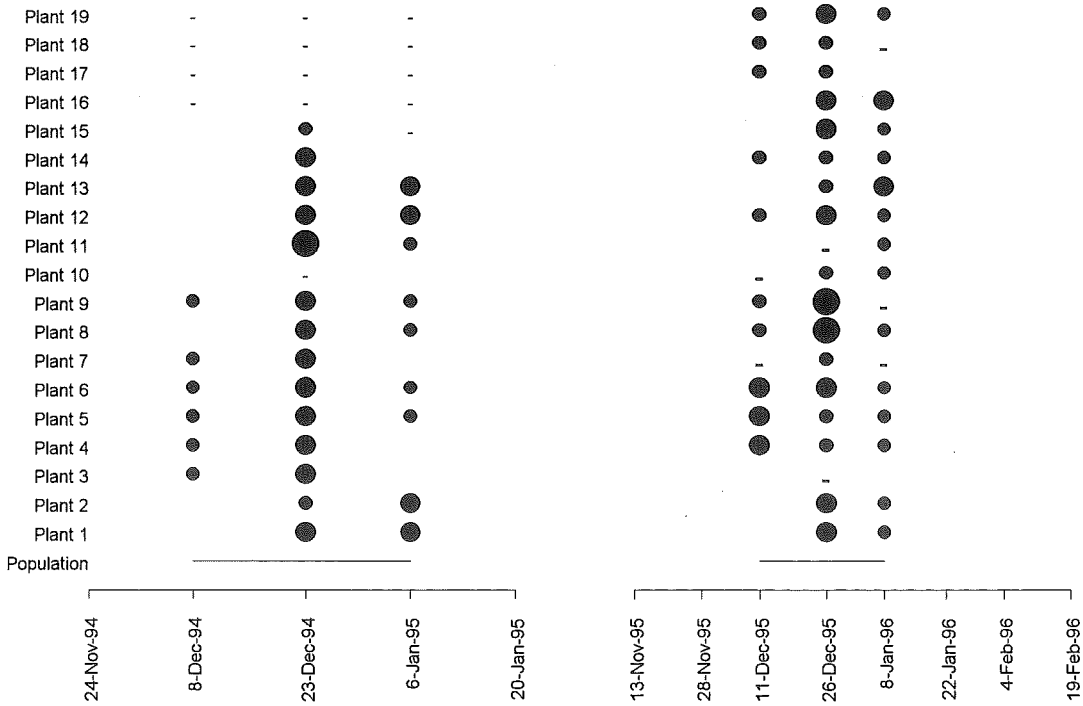


Figure 3.17: The flowering phenology of 19 tagged individuals of *Raoulia australis* at Broad Stream over the 1994/95 and 1995/96 season. (See Figure 3.8 for explanation of symbols.)

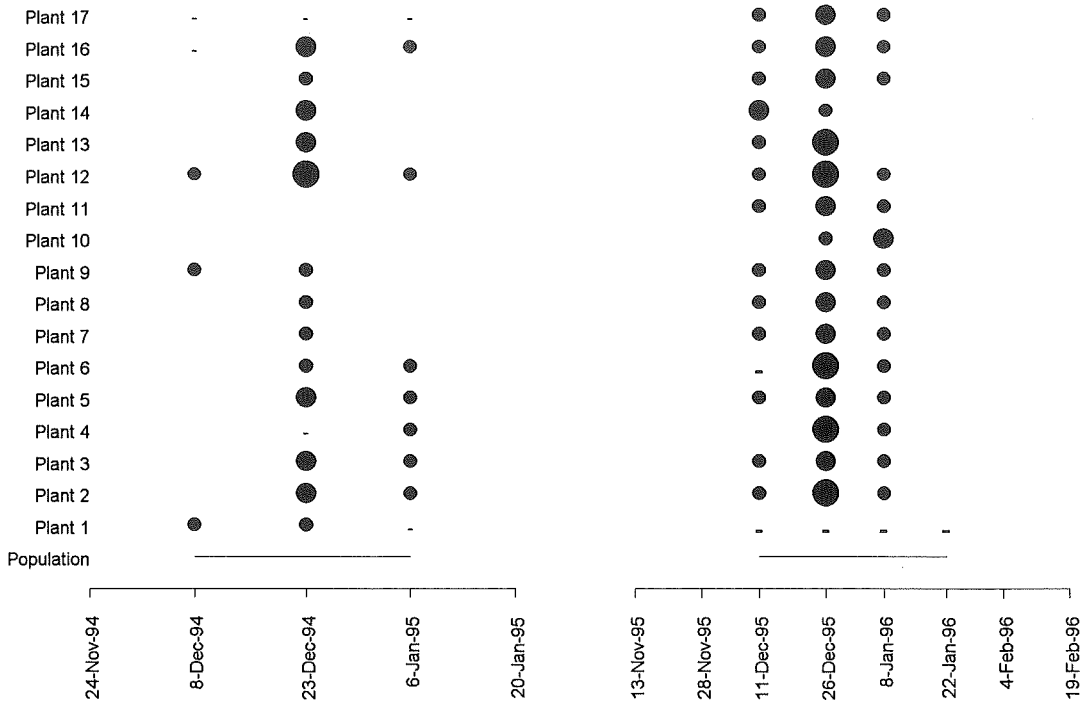


Figure 3.18: The flowering phenology of 17 tagged individuals of *Raoulia australis* at the Cass River over the 1994/95 and 1995/96 season. (See Figure 3.8 for explanation of symbols.)

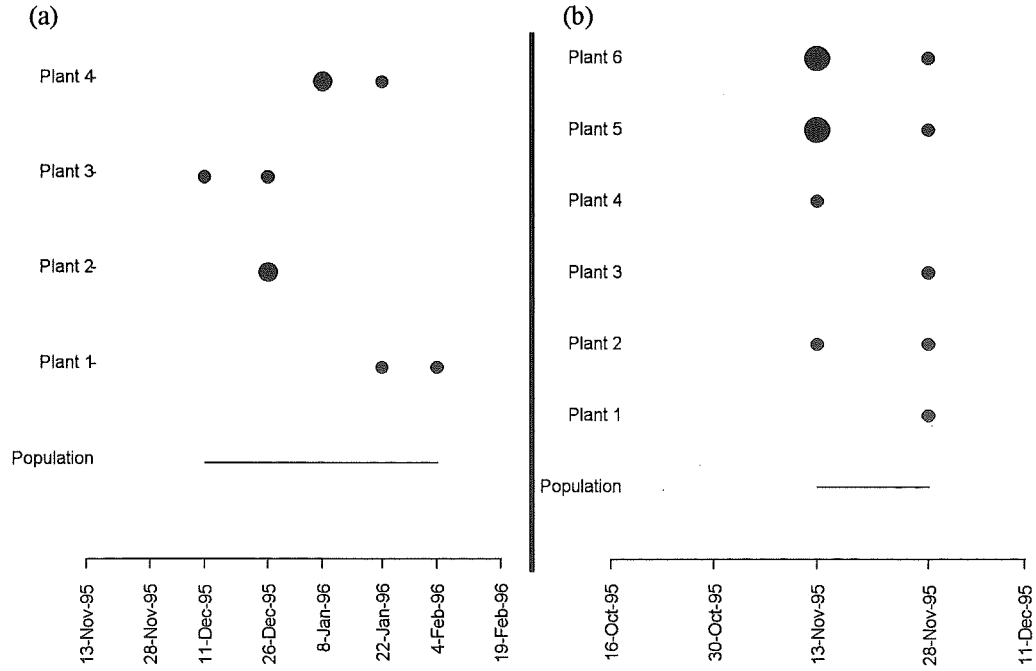


Figure 3.19: The flowering phenology of tagged individuals of (a) *Raoulia australis* and (b) *R. haastii* at Dry Stream during the 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

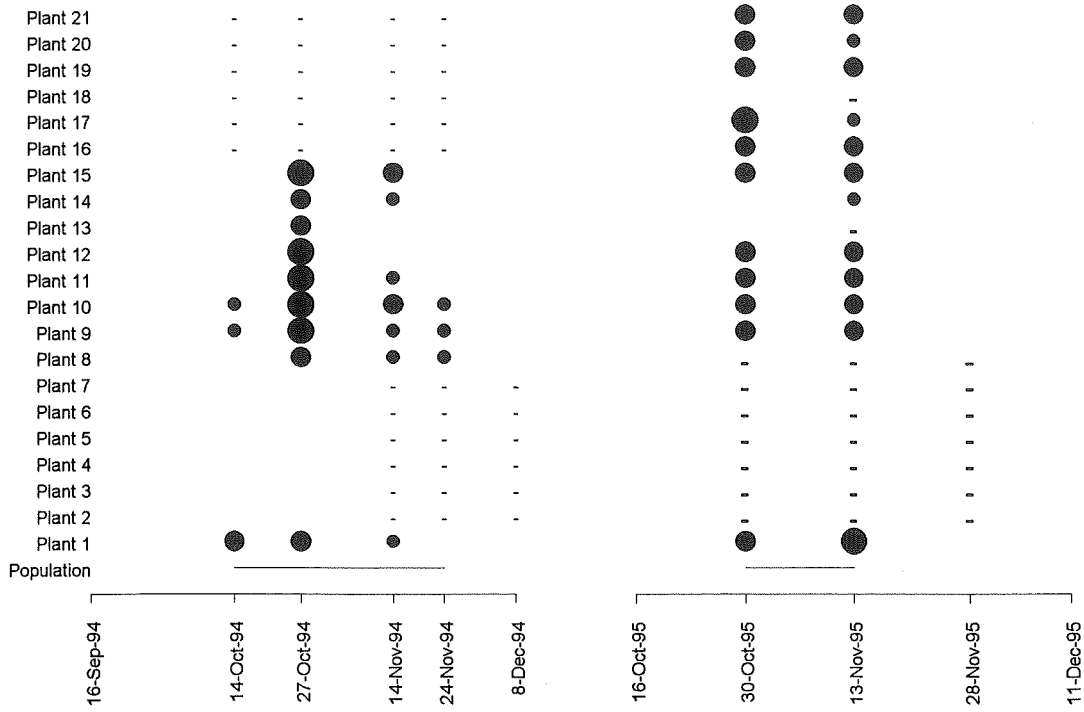


Figure 3.20: The flowering phenology of 21 tagged individuals of *Raoulia haastii* at Broad Stream over the 1994/95 and 1995/96 season. (See Figure 3.8 for explanation of symbols.)

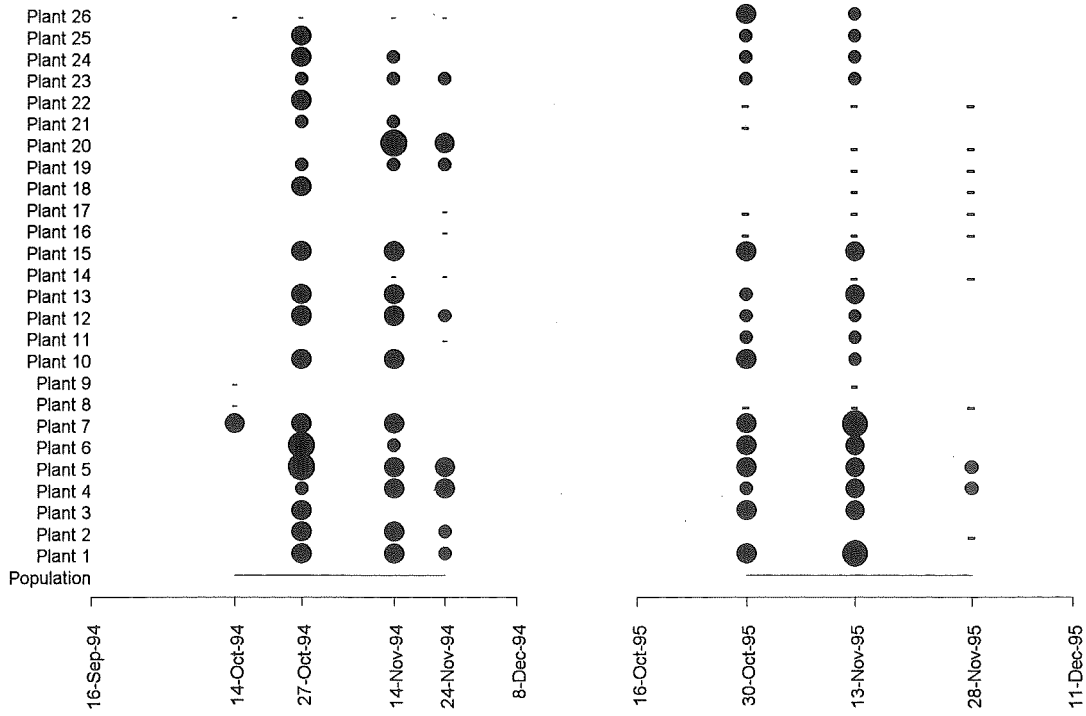


Figure 3.21: The flowering phenology of 28 tagged individuals of *Raoulia haastii* at the Cass River over the 1994/95 and 1995/96 season. (See Figure 3.8 for explanation of symbols.)

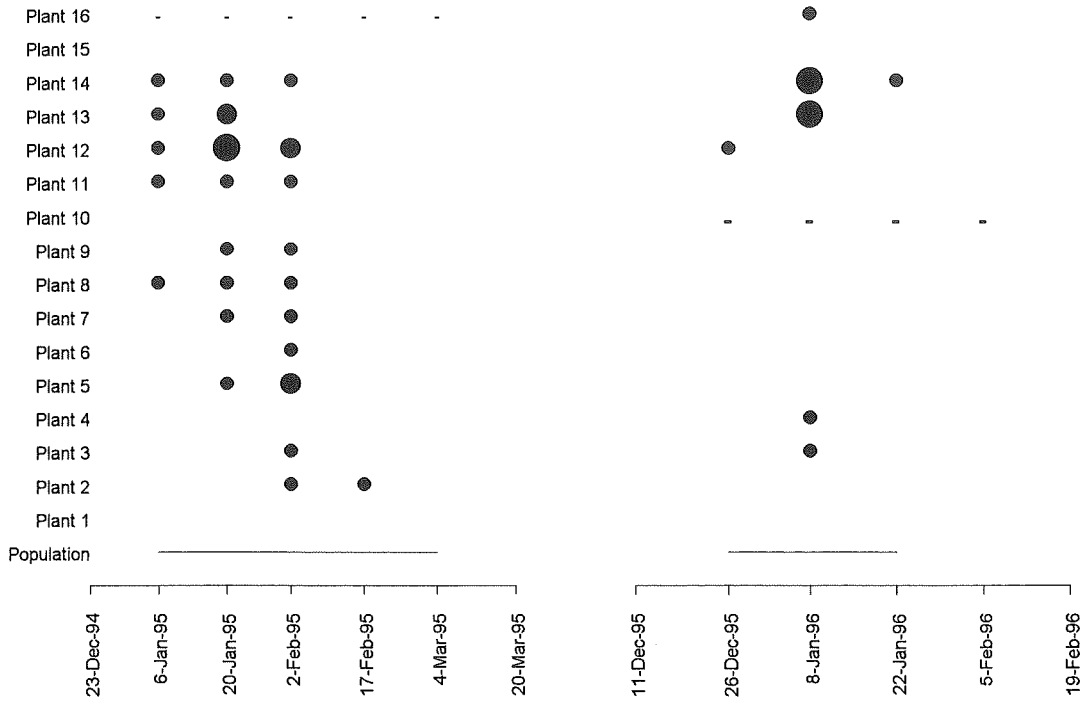


Figure 3.22: The flowering phenology of 16 tagged individuals of *Raoulia grandiflora* at Broken River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

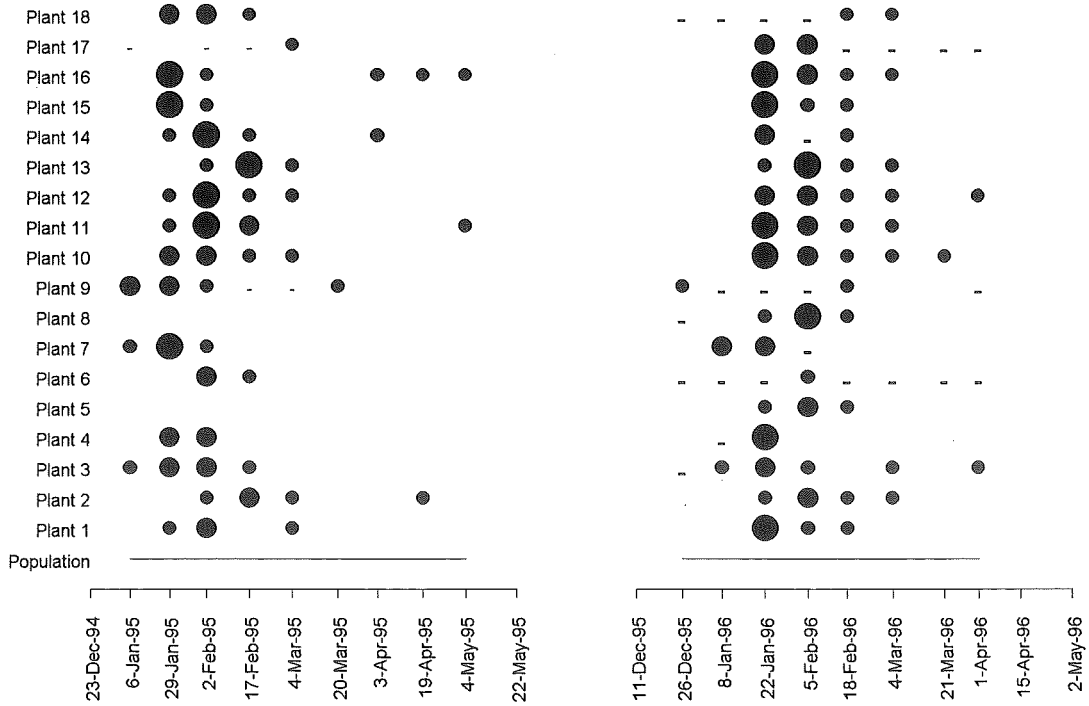


Figure 3.23: The flowering phenology of 18 tagged individuals of *Raoulia hookeri* at Broad Stream over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

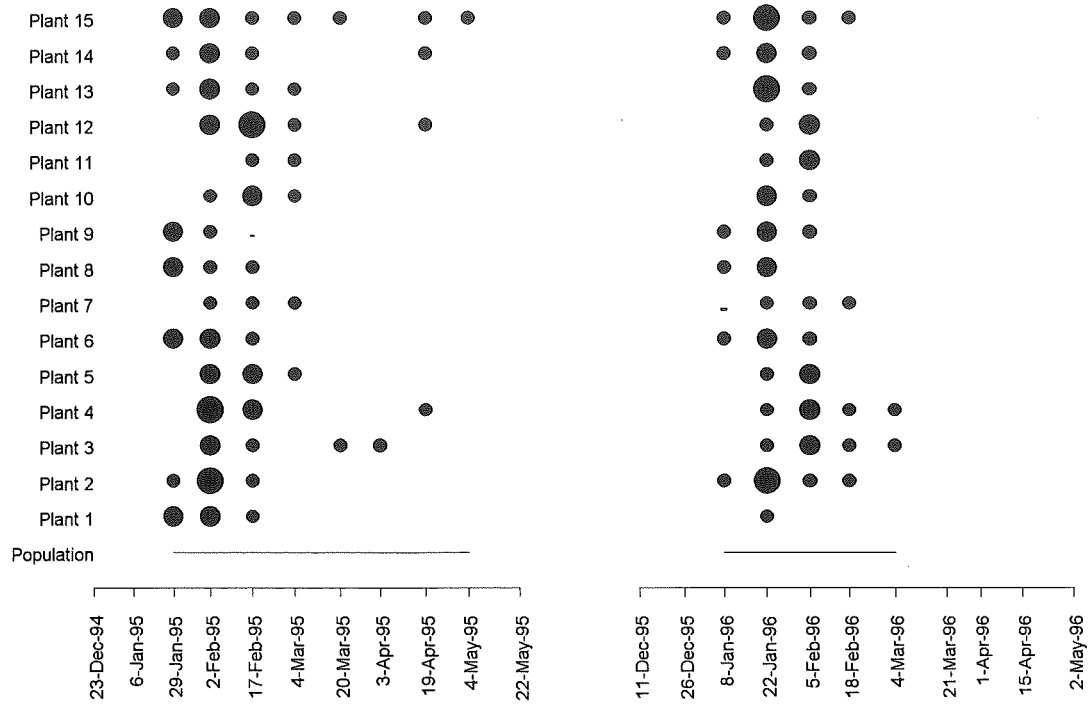


Figure 3.24: The flowering phenology of 15 tagged individuals of *Raoulia hookeri* at the Cass River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

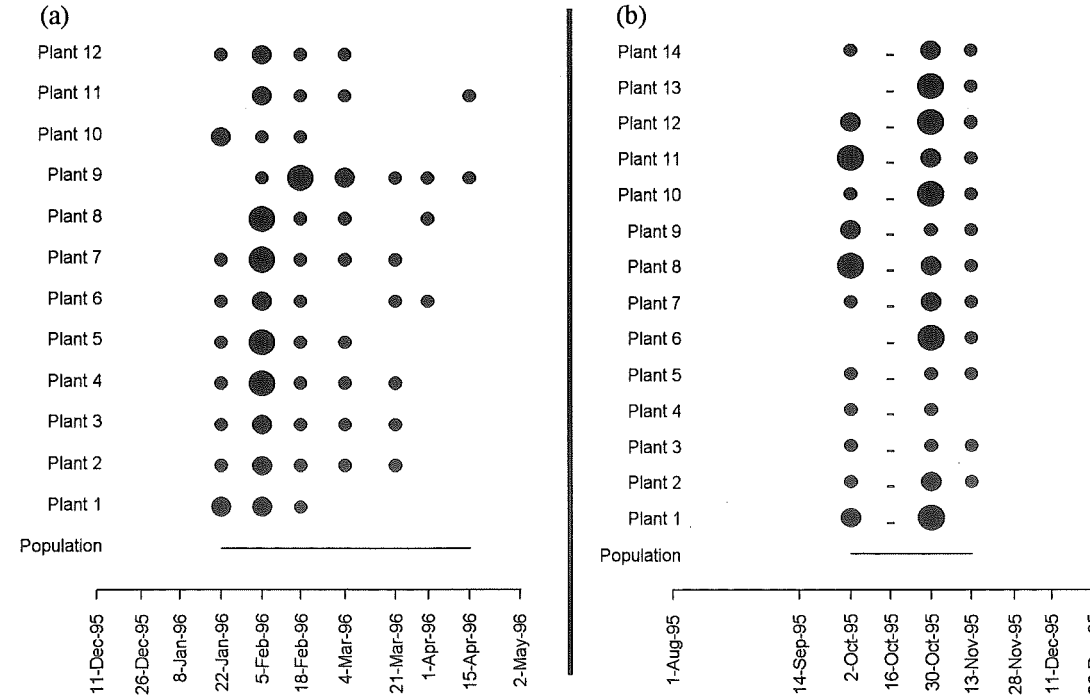


Figure 3.25: The flowering phenology of tagged individuals of (a) *Raoulia hookeri* and (b) *R. tenuicaulis* at Dry Stream over the 1995/96 season. (See Figure 3.8 for explanation of symbols.)

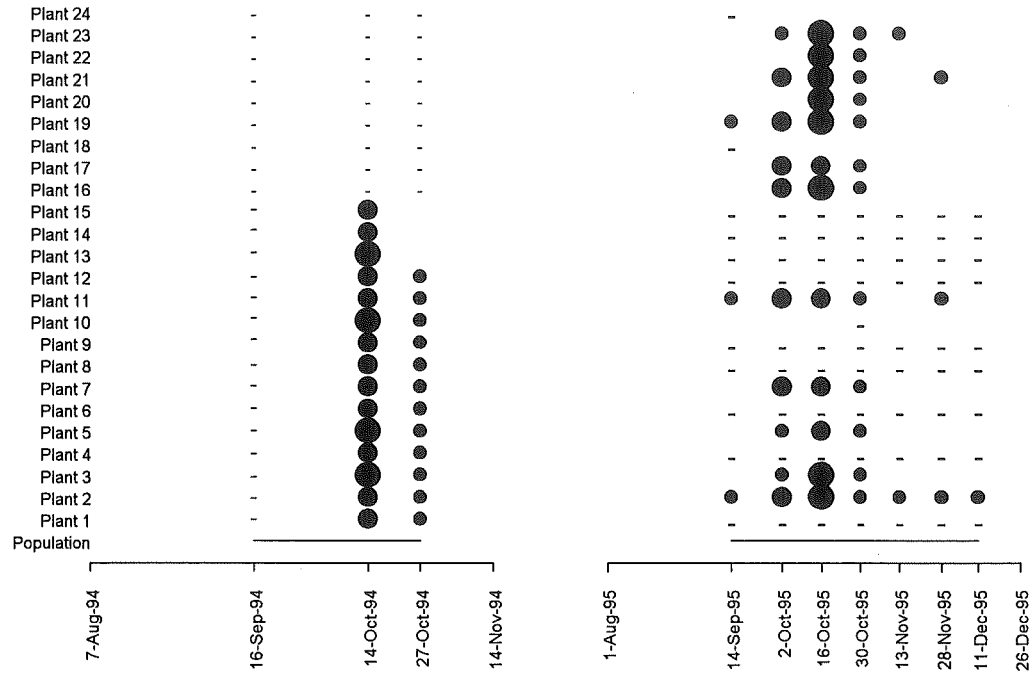


Figure 3.26: The flowering phenology of 24 tagged individuals of *Raoulia tenuicaulis* at Broad Stream over the 1994/95 and 1995/96 season. (See Figure 3.8 for explanation of symbols.)

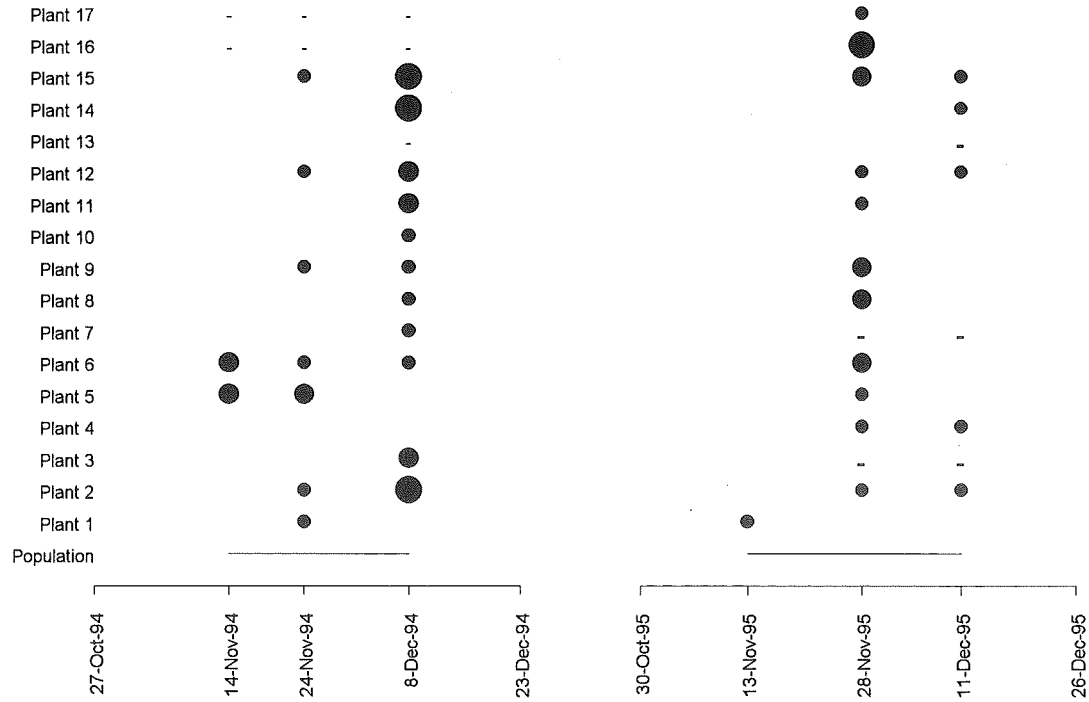


Figure 3.27: The flowering phenology of 17 tagged individuals of *Raoulia monroi* at Cass over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

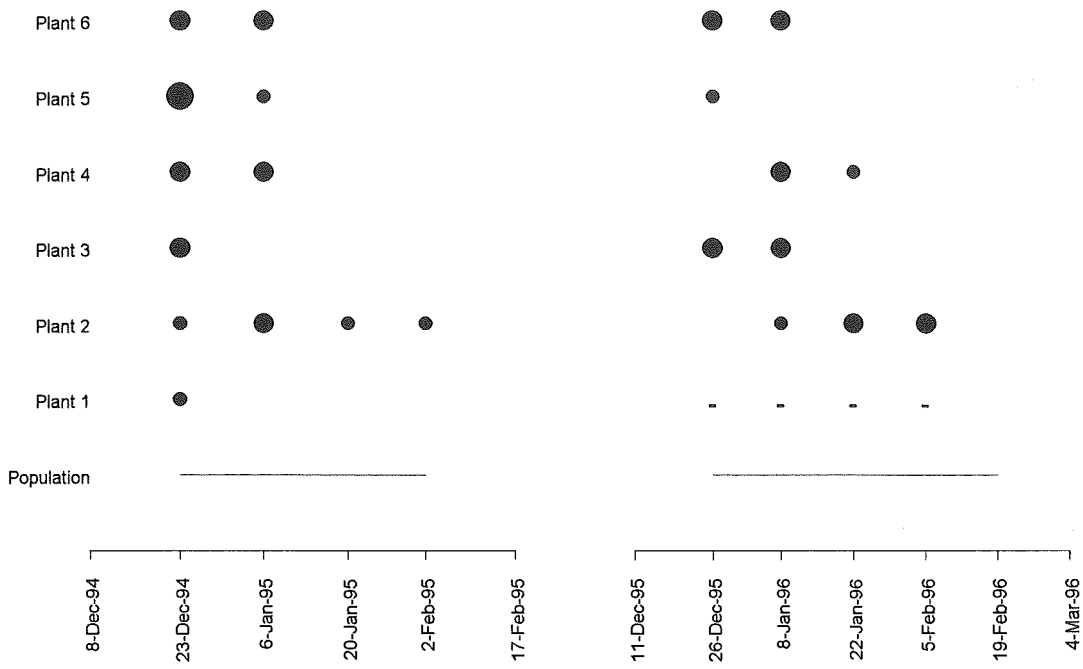


Figure 3.28: The flowering phenology of 6 tagged individuals of *Raoulia mammillaris* at Broken River over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

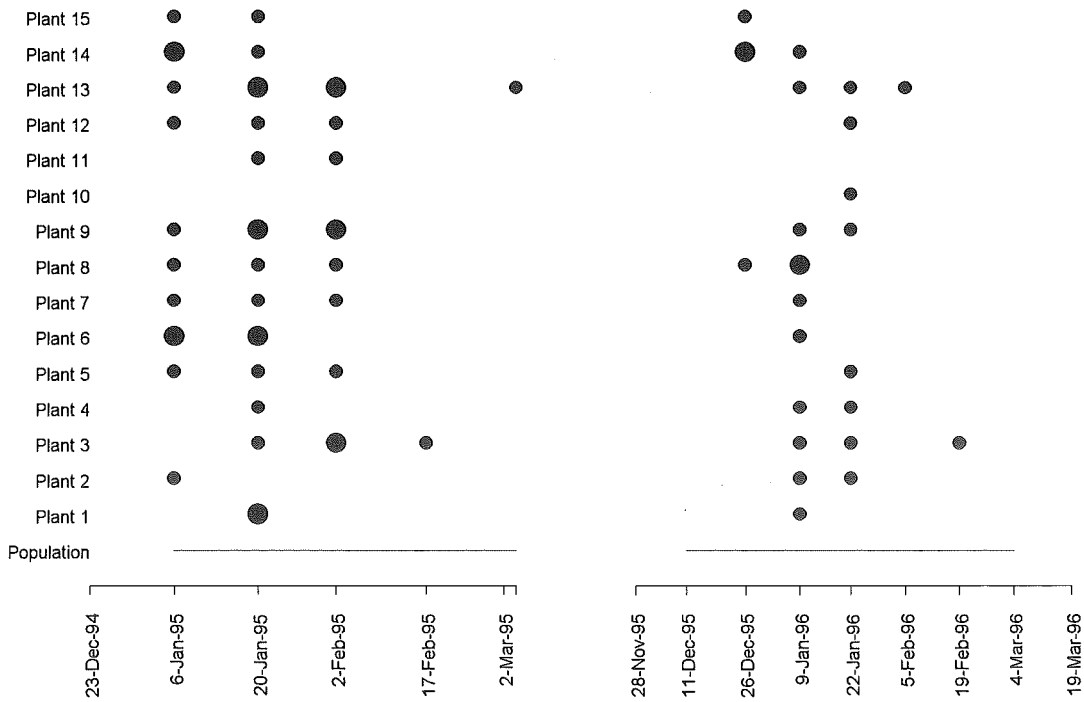


Figure 3.29: The flowering phenology of 15 tagged individuals of *Raoulia subsericea* at Cass over the 1994/95 and 1995/96 seasons. (See Figure 3.8 for explanation of symbols.)

3.4.3 Cluster phenology

Gnaphalium audax: The central capitulum reaches anthesis first, and is usually in late anthesis when the next capitula reach anthesis. Each capitulum generally opens when the previous capitulum is in late anthesis, with the sequence of opening generally being basipetal. Usually not more than two capitula open at any one time. The central capitulum will often begin seed dispersal before all capitula in a cluster reach anthesis, and each subsequent capitulum releases its seed as soon as the seed is mature.

Leucogenes grandiceps: The central capitulum is always the first to reach anthesis, unless it is subject to predation by Tephritid fly larvae. In all cases of predation that were observed in *L. grandiceps*, the central capitulum was the only capitulum in the cluster to be affected. The central capitulum may reach anthesis when the outer, fleshy bracts have not fully spread, and are still covering the outer capitula. The capitula open basipetally, however, the timing of the start of anthesis of the outer capitula is slightly variable. The first outer capitula begin to open when the central capitulum has a only few, or, up until approximately half of the tubular florets at anthesis. The outer capitula may open in quick succession, or be staggered so that the last capitulum to open will only have filiform florets at anthesis when the last tubular floret in the central capitulum reaches anthesis. The capitula end anthesis in the order they opened.

Ozothamnus leptophyllus: There appears to be no fixed pattern to the cluster phenology of *O. leptophyllus*. The only discernible pattern was for the capitula on the north facing side of the clusters to open before the capitula on the more shaded, southern side.

3.4.4 Capitula phenology

Descriptions from Field Material

The following terms are used in the descriptions of the capitula phenologies. The first tubular floret(s) to open (i.e. reach anthesis) are referred to as the "initial" tubular floret(s), while later opening florets are referred to by the term "subsequent". The end of anthesis of all florets is indicated by the browning of the style arms and corolla, the term "brown" or "browning" is used to refer to this phenomenon. Florets opening, and entering the different

stages of anthesis at the same rate, are referred to as a "group". The "inner florets" are those close to the centre of the capitulum, while the term "outer florets" refers to those near the periphery. When an angle of the florets or involucral bracts is given the number of degrees indicates the angle the structure has reflexed from the vertical. The pappus hairs are referred to simply as pappus.

Development of the capitula in all species is centripetal, with the possible exception of *Raoulia haastii* in which at least one of the tubular florets opens first. The filiform florets, which are structurally and functionally female, are placed at the periphery of the capitulum, and begin presenting their styles as soon as they reach anthesis. The tubular florets are protandrous, presenting pollen at the end of the stamen tube soon after the floret reaches anthesis. Following pollen presentation, the stamen tube withdraws or splits, and in the tubular florets of most species the style is presented. This pattern is detailed more fully in section 3.4.5 (Floret phenology).

The average number of filiform (F) and tubular (T) florets in the capitula of each species are presented following the species name to provide a reference to the capitula sizes. These values are presented in full in Table 3.2.

Gnaphalium audax (F: 51.0, T: 3.7)

The capitula are initially tightly closed giving the bud a pointed appearance. As the bud begins to develop and enlarge, the involucral bracts begin to separate at the top. The involucral bracts continue to widen so that the top of the capitulum takes on a flatter appearance. When only a small gap has appeared between the top of the involucral bracts the styles of the first filiform florets become visible extending just above the top of the involucral bracts (e.g. Plate 19A). As anthesis continues the styles of the filiform florets continue to extend, and begin to curl. Once all the filiform florets are at anthesis the top of the capitulum is a mass of styles of filiform florets. At this stage the first of the tubular florets becomes visible, and rapidly opens to present pollen. The first tubular floret(s) to open may occur singly or as a pair. Although the first tubular floret(s) usually appear at the stage described above, occasionally the tubular florets begin anthesis shortly after the first filiform florets are at anthesis, so that the styles of the filiform florets only just protrude above the involucral bracts, and have not yet begun to curl. The initial tubular florets are presented below the level of the involucral bracts. However, as more open they become

increasingly raised so that the later opening tubular florets are presented level with, or just above, the involucral bracts. The subsequent tubular florets open singly or occasionally in pairs (e.g. Plate 19B), when the previous tubular has entered the female phase. Each of the tubular florets remains in the female phase for a only brief period, such that the style will have withdrawn by the time the subsequent tubular floret(s) enters the female phase.

The browning of florets in *G. audax* shows two patterns. In the first pattern the filiform florets begin to brown when two or three tubular florets have opened, and all are brown before the last tubular floret(s) opens. However, more usually a few filiform florets at the outside of the capitulum remain open until after all other florets have finished anthesis. The majority of filiform florets brown before all the tubular florets have reached anthesis. Once the filiform florets begin to brown, the number that remains at anthesis declines rapidly. As they brown, the styles of filiform florets withdraw. Typically the last florets at anthesis are a few outer filiform florets.

Gnaphalium traversii (F: 121.0, T: 7.8)

The stages of capitula phenology are the same as *G. audax*, except that up to three tubular florets may open in a group.

Helichrysum bellidioides (F: 97.4, T: 82.9)

The involucral bracts open progressively over a series of days, until they are well spread. When the involucral bracts are well spread, the filiform florets begin to open, presenting their styles while still well below the top of the pappus (Plate 20A). The filiform florets open rapidly, and are usually all open, or nearly all open, when the first tubular floret(s) becomes visible. As anthesis continues, the style arms of the filiform florets gradually spread, and the styles elongates so that the arms are presented above the pappus. Occasionally a tubular floret will open and begin presenting pollen before all the filiform florets are open, sometimes when only 10-15 filiform florets are open. However, the usual pattern is for the first tubular floret to open just after, or at the same time, as the last filiform floret opens. By this stage the styles of the first filiform florets to open are clear of the pappus, and their style arms moderately well spread (Plate 20B). The initial tubular floret(s) opens in a group of one to three, however usually this is followed rapidly by a larger group of tubular florets, so that as many as 10 florets may form an initial group. The tubular florets continue opening, with each new group opening as the previous group enters

the female phase. The tubular florets continue opening in this pattern until all are open (Plate 20D). The group size of the tubular florets varies, with groups as large as 12 florets occurring early in anthesis, while the later groups (towards the centre of the capitulum) are usually smaller (i.e. four or five tubular florets per group). The last tubular floret(s) to open occurs singly or as a pair. The later tubular florets to be open, are presented at a higher level than the earlier florets, so that the capitulum has a dome shaped appearance (Plate 20E).

The timing of browning in *H. bellidioides* is variable, but generally follows one of three broad patterns. These are:

(1) When all, or nearly all, of the florets are open, the florets begin to brown starting at the outside first, with the outer tubular floret starting at the same time as the filiform florets. In this pattern, the filiform and outer tubular florets are brown well before all the inner most tubular florets.

(2) The florets begin to brown well before all the florets have reached anthesis, such that only the two or three groups of florets will be at anthesis at one time, i.e. the only florets at anthesis will be the tubular florets presenting pollen and the group or two groups immediately preceding those presenting pollen. The filiform and outer tubular florets brown before half of the tubular florets are open.

(3) Browning occurs in an intermediate pattern to (1) and (2), such that the florets begin to brown when approximately 15-20 tubular florets still have not opened. A greater number of florets remain at anthesis than in pattern (2).

In all three patterns the last floret to brown is the central tubular floret (Plate 20F).

Helichrysum depressum (F: 0, T: 11.3)

The involucral bracts are initially tightly closed. As they begin to open, the pappus becomes visible between the top of the involucral bracts. When first visible, the pappus tips are well below the top of the involucral bracts. However, as the involucral bracts open further the pappus appears to extend, so that by the time the first florets open the pappus is just taller than the involucral bracts. When fully mature the top of the involucral bracts are slightly angled outward (Plate 22C). The tubular florets were the only type of floret observed in *H. depressum*. The florets become visible between the pappus hairs while still well below the top of the pappus. When the florets are level with the top of the pappus, the corolla splits and the florets rapidly begin to present pollen. The initial group of florets

contains between one to five tubular florets (Plate 22A), while subsequent groups are generally composed of one to three (Plate 22B), or occasionally, four florets. Each group begins presenting pollen when the previous group is in early to mid female phase, although occasionally the next group may not open until the previous group has reached the late female phase. The last floret to open is usually a single tubular floret in the centre of the capitulum. The outer florets often begin to brown before all the florets are open, so that by the time the last tubular floret in the female phase, approximately half of the florets will be brown. A less frequent pattern of browning occurs when all of the florets remain at anthesis until all the florets are open. The florets then usually brown in the order they opened (Plate 22C).

Helichrysum filicaule (F: 15.4, T: 20.9)

The capitulum is initially visible as a small, tight bud. The bud gradually enlarges as it develops, and when nearly mature, the top of the pappus hairs become visible between the top of the involucral bracts. Initially the pappus is only minutely visible, but as the capitulum matures further the pappus becomes more prominent. When first visible the pappus hairs are below the top of the involucral bracts; however by the time the first floret opens, the tips of the pappus hairs are taller than the involucral bracts. The florets become visible through gaps in the pappus hairs shortly before they open. The first florets to open are the filiform florets. These are presented initially between the involucral bracts and the pappus hairs, or in gaps between the involucral bracts (Plate 21A). The filiform florets open in quick succession, so that by the time the first tubular floret opens, all the filiform florets are at anthesis, with their style arms slightly spread (Plate 21B). By this stage the corolla of the filiform florets is usually visible just above the pappus, or between the involucral bracts. Occasionally some filiform florets finish anthesis by the time the first tubular floret opens. The initial tubular floret occurs singly, or in a group of up to 15 florets. The subsequent tubular florets appear to open in large groups during early anthesis, while later groups usually only contain four or five tubular florets per group. Each group opens, and begins presenting pollen when the previous group is in the early female phase (Plate 21C). The last tubular floret to reach anthesis usually opens as part of a group of two or three florets. As anthesis progresses the outer florets gradually bend outward so that the capitulum alters from having a flat topped appearance, to having a more round appearance (Plate 21A, C, D, E). The later opening florets are also presented at a slightly higher level than the earlier florets.

The timing of browning in *H. filicaule* is variable. As indicated above, some filiform florets may begin browning before the first tubular floret opens. The tubular florets then brown in approximately the same order in which they opened, with only two or three groups of tubular florets at anthesis at one time. This pattern of browning was the most frequently observed. An alternate pattern of browning occurs when the filiform florets do not brown until nearly all of the tubular florets are open. When this occurs the style arms of tubular and filiform florets curl extensively (Plate 21F) before the filiform and outer tubular florets begin browning. The order of tubular floret browning is less organised than in the first pattern, but still generally occurs in the earlier opening florets first. The last floret to brown in both patterns is usually the central tubular floret.

Helichrysum intermedium (F: 10.8, T: 23.9)

The buds are initially tightly closed. As the bud matures, the involucral bracts begin to open. The involucral bracts continue to open, and gradually bend throughout anthesis, so that by the end of anthesis, the involucral bracts have bent outwards to 90°, or more. By the time the involucral bracts are open vertically, the tops of all the florets are visible between the pappus. The filiform florets are first to reach anthesis, each floret opening when the top of the floret is just above the level of the pappus (Plate 23A). The filiform florets open in quick succession, and are all opening and presenting their style before any of the tubular florets reach anthesis. The first tubular floret(s) open when the styles of the filiform florets are extended to approximately half of their full extension. Each tubular floret opens when the top of the corolla is above the pappus hairs. The tubular florets open in groups of one to four, with each subsequent group beginning to open when the previous group enters early female phase. The last floret to reach anthesis is the central tubular floret, which may open singly or as one of a pair. As the capitulum develops, the florets gradually bend towards the outside of the capitulum, especially the outer florets (Plate 23B). Eventually, the filiform and outer tubular florets bend outward to 90°, or more (Plate 23C). As the subsequent tubular florets open, their corolla lobes overlap those of the previous florets, so that the earlier florets may be difficult to see, especially the filiform florets. Browning usually begins before all the florets are open. The first florets to brown are usually the filiform florets, closely followed by the outer tubular florets. These florets usually start to brown when 10 to 16 tubular florets are still not at anthesis, although one or two individual florets may brown very early in anthesis. For example, one or two filiform

florets sometimes brown when only 5 to 10 tubular florets are at anthesis. The last florets to brown are the central tubular florets.

Leucogenes grandiceps (F: 10.4, T: 19.2)

When the capitulum first become visible between the fleshy bracts, the involucral bracts are tightly closed together. As the capitulum matures, the involucral bracts gradually open, revealing the pappus. The filiform florets are the first to reach anthesis, with their corollas opening when the top of the floret is just below the level of the pappus. All the filiform florets open and begin to present their styles in quick succession. Initially, the style arms of the filiform florets are oriented radially. The first tubular floret usually opens when all the filiform florets are at anthesis (Plate 19C), however, the first tubular floret(s) will sometimes reach anthesis before all of the filiform florets are open. The initial tubular floret opens in a group of one to three florets. Subsequent tubular florets open in groups of two to six, except for the last floret, which usually occurs individually. Subsequent groups of tubular florets appear to open when the previous group has reached early to mid female phase. When each group opens the tops of the florets in the next group are visible between the pappus hairs. The filiform florets begin to brown before all the tubular florets are open, usually when the capitulum is half to two thirds of the way through anthesis. Usually the filiform florets are completely brown before the first tubular florets begin to brown. Occasionally, however, a few tubular florets will begin to brown at about the same time as the filiform florets. These tubular florets usually appear to brown more slowly than the filiform florets. The tubular florets usually begin browning from the outside of the capitulum first, with at least 5 to 10 tubular florets normally brown by the time the last floret reaches anthesis. The last florets to brown are the central tubular florets.

Ozothamnus leptophyllus (F: 0, T: 7.3)

The buds are initially very tightly closed, with only the bronze coloured outer involucral bracts visible. As the bud enlarges, the tip of the inner, white involucral bracts become visible (Plate 24A, capitulum in bottom centre). As the bud develops further, the white involucral bracts extend further above the bronze involucral bracts, and gradually begin to separate (Plate 24A), exposing the pappus. The involucral bracts continue to open, and eventually start to bend so that by mid anthesis the involucral bracts are presented at 90° or more (Plate 24F). Soon after the pappus becomes visible, the top of the first floret becomes visible below the top of the pappus. The first floret to open is usually a single

tubular floret (Plate 24C), however occasionally, a group of two to three florets may form the initial group. Subsequent groups of one to three florets usually become visible as the previous group enters early female phase (Plate 24D). The last floret to open usually occurs singly (Plate 24F). The florets often begin to brown early in the anthesis of the capitulum, so that only two or three groups of florets are at anthesis at any one time. Alternatively, the florets may not begin to brown until all florets are open, except for two or three central florets. The florets usually brown in the order in which they opened.

Raoulia australis (F: 3.4, T: 5.1)

As the capitulum develops the involucral bracts gradually separate, but remain closely pressed against the florets throughout anthesis. The filiform florets are the first to reach anthesis, presenting their styles while the involucral bracts have only just separated at the tips (Plate 25A). All the filiform florets appear to reach anthesis at the same time. Initially, only the tips of the styles are visible, pressed between the involucral bracts and the pappus, however as the florets develop the corolla becomes minutely visible. The styles of the filiform florets appear to elongate during anthesis, so that by the time the top of the first tubular floret is visible, the style arms are spread horizontally, and spread to approximately half their maximum extension. The initial tubular floret is first visible pressed between the pappus and the involucral bracts. By this stage the involucral bracts are approximately vertical. The initial tubular floret usually appears singly, although occasionally two may open together (Plate 25C). Subsequent groups of tubular florets usually contain one or two florets per group. While the current group of tubular florets is still presenting pollen the top of the next group of becomes visible. By the time the previous group of tubular florets is reaching the end of pollen presentation, the corollas of the next group are just beginning to split, so that pollen presentation in two sequential groups will overlap slighlty (Plate 25B). This pattern of emergence continues until all tubular florets are open (Plate 25D). The last floret to reach anthesis is usually a single tubular floret. By the time the last tubular floret opens, the styles of the filiform florets are fully extended, and usually extensively curled. Occasionally a single filiform floret may brown before all the tubular florets reach anthesis, however the filiform florets usually begin browning only after all the florets in the capitulum are open. The filiform styles are usually the first floral part to brown, followed by the corollas of the filiform and tubular florets, and lastly by the styles of the tubular florets. Occasionally, however, a filiform will continue to present its style after all the other florets have finished anthesis.

Raoulia glabra (F: 13.3, T: 30.9)

The capitula are initially tightly closed buds, with the top of the involucral bracts overlapping (Plate 19E). The involucral bracts gradually open, exposing the pappus. As anthesis continues, the involucral bracts gradually bend outwards, so that by the time approximately half the tubular florets are open, the involucral bracts are bent out to approximately 90°. Once visible the pappus appears to elongate, and gaps appear in the pappus exposing the tops of the tubular florets. The filiform are the first florets to reach anthesis, becoming visible just before the corolla opens. At this stage the tops of the filiform florets are well down the side of the pappus, and the involucral bracts have spread so they are presented vertically, or just past vertical. The filiform florets appear to reach anthesis in a slightly staggered arrangement, however nearly all filiform florets are open and presenting their styles before the first tubular floret opens. The styles of the filiform florets continue to lengthen throughout anthesis, so that by the time approximately 10 tubular florets are at anthesis, the junction between the style arms of the filiform florets are level with the top of the pappus. At this stage the top of the filiform corolla is also visible. The first tubular floret begins to present pollen when the tips of the filiform style arms are approximately level with the top of the pappus. The tubular florets appear to develop in quick succession with one to five florets per group, although most groups usually contain three tubular florets. The last floret to open usually occurs singly. The next group of tubular florets appear have their stamen tubes well extended, or are presenting pollen, when the previous group of florets enters the female phase. As the anthesis of the capitulum progresses, the florets (especially those to the outside) bend so that the styles usually extend out beyond the bract tips. In addition, the tubular florets appear to open at increasing higher levels, so that by the end of anthesis the capitulum has changed from an approximately flat surface at the start of anthesis, to a dome shaped surface. The florets begin browning before all the tubular florets have reached anthesis, with the outer tubular and filiform florets beginning to brown at about the same time. The last floret to brown is usually the central tubular floret.

Raoulia grandiflora (F: 11.6, T: 20.2)

The involucral bracts initially form a tightly closed bud. The involucral bracts gradually open throughout anthesis, achieving their maximum spread when the second or third group of tubular florets is at anthesis. When the involucral bracts are spread to approximately

35°, the filiform florets become visible as pairs of style arms between the pappus hairs. The style of the filiform florets elongate throughout anthesis, so that if they have not browned by the time approximately half of the tubular florets are open, they are presented well clear of the pappus. The tubular florets become visible while still well below the level of the pappus. The first tubular floret reaches anthesis when the filiform styles are presented just above the level of the pappus (Plate 26D). All the tubular florets open in groups of one to four, with the next group opening when the previous group of tubular florets is in the early female phase. The number of tubular florets in each group tends to decrease as anthesis progresses, so that the last few florets are usually presented singly. Browning of the florets is variable. The filiform florets may brown before any of the tubular florets open, or more commonly, may start browning when 8 to 10 tubular florets have reached anthesis. The filiform florets always appear to brown first, followed by the outer tubular florets. Usually the first tubular floret begins to brown at the same time as the last filiform floret. The first tubular floret is therefore usually completely browned before all the tubular florets are open.

Raoulia haastii (F: 2.3, T: 2.0)

The involucral bracts of *R. haastii* do not spread widely during anthesis, remaining tightly closed around the florets. Therefore, the swollen tops of the florets become visible when the florets are already well developed, just prior to the corolla opening. The first floret to reach anthesis is usually a single tubular floret (Plate 27A), although occasionally two tubular florets may open initially. (In only one plant, located at Broad Stream, were the filiform florets observed to open at the same time as the first tubular floret(s). In addition, only a few capitula on this plant varied from the normal pattern of presenting only tubular florets initially.) Soon after the top of the initial floret becomes visible, the corolla splits, and the floret begins to present pollen. When this floret is nearing the end of pollen presentation, or has entered the female phase, the styles of the filiform florets become visible pressed between the open tubular floret(s) and the involucral bracts. Once they are visible the style arms of the filiform florets begin to elongate and curl. The corolla also appears to elongate so that soon after the style arms are visible, the corolla of the filiform florets also becomes visible. The second tubular floret usually becomes visible after the filiform florets have reached anthesis, when the first tubular floret is usually in the early female phase (Plate 27C, top left capitulum). Once visible this floret opens rapidly, and begins to present pollen (Plate 27B; 27C, bottom right capitulum). At the point at which

the second tubular floret enters the female phase, the style of the filiform florets and the initial tubular floret(s) are fully extended, and usually extensively curled (Plate 27D).

The first florets to brown are usually either the filiform florets, or the first tubular floret(s) to open. As the florets brown, the corolla and styles withdraw below the level of the involucral bracts (Plate 27E). The last tubular floret to reach anthesis also appears to be the last floret to brown. In a few capitula (estimated at less than 1 per 100) a single tubular floret may open and begin presenting pollen, after all the other florets have completed anthesis. The stamen tube and styles of these late florets do not extend very far beyond the top of the involucral bracts (Plate 27E, bottom right).

Raoulia hookeri (F: 9.0, T: 11.3)

The involucral bracts are initially tightly closed. The involucral bracts continue to straighten, until they are completely upright. As the involucral bracts begin to open the pappus is exposed. As the capitulum ages, the pappus in the centre of the capitulum becomes slightly raised above the height of the adjacent outer pappus. When the involucral bracts are nearly upright, the tips of the filiform style arms become visible. By the time the involucral bracts are upright all the filiform florets are open, and have their style arms well spread, just above the level of the involucral bracts and pappus. The filiform florets appear to open in quick succession, however there is usually a slight delay between the opening of the first and last filiform florets. When first presented the style arms of the filiform florets are oriented radially. The corolla of the filiform florets varies as to whether it is visible during anthesis; in some plants the corolla will be visible just above the level of the involucral bracts, while in others the corolla remains below the level of the involucral bracts throughout anthesis. Shortly before the tubular florets appear, gaps start to develop in the pappus. The initial tubular floret opens in a group of one to five, with four florets being the usually number of tubular florets in the initial group. The next group of tubular florets open when the previous group has entered the "female" phase. (The tubular florets are functionally male.) The last floret to open always appears to occur singly. The first floral part to brown usually appears to be the corollas of the tubular florets, although occasionally a style of a filiform floret may brown first. When the corollas of the tubular florets have browned, any remaining styles of the filiform and outer tubular florets brown. The styles of the central tubular florets are last to brown.

Raoulia mammillaris (F: 4.4, T: 6.6)

When involucral bracts have opened, and are positioned approximately vertical, the filiform florets become visible well down the side of the pappus. Soon after they first become visible the filiform florets open, and the styles begins to elongate and spread (Plate 26C, capitulum on right). The filiform florets appear to open in a slightly staggered arrangement, so that by the time the last filiform floret reaches anthesis, the style of the first filiform floret to open has elongated and spread slightly. When all the filiform florets are open, and presenting their styles, the tops of the outer tubular florets are visible level with the pappus tips. By the time the first tubular floret opens, the styles of the filiform florets are well spread, and the corolla has elongated so that it is usually visible level with the top of the pappus. The first tubular floret usually opens as part of a group of one to three florets (Plate 26A; 26C, left capitulum), with the florets opening when the top of their corolla is above the level of the pappus. The next group of tubular florets begins to open, and has its stamen tubes well extended when the previous group is in mid female phase (Plate 26B), or occasionally when the previous group of tubular florets is still presenting pollen. The tubular florets continue to open in this pattern, in groups of two or three, until all florets are open. The last floret to open is usually a single tubular floret. Normally the styles of the filiform florets are first to brown, followed by the tubular corollas and styles. However, occasionally the corollas of the tubular florets will begin to brown first, followed by the styles of the filiform, then the tubular, florets. Usually the first florets begin to brown before the last tubular floret opens, so that the styles of the filiform and one or two tubular florets are usually completely brown before the last tubular floret opens.

Raoulia monroi (F: 4.7, T: 8.0)

The involucral bracts initially form a tightly closed bud. As the involucral bracts separate, the dense pappus is exposed. As the involucral bracts spread further, the pappus appears to elongate, so that it becomes taller than the involucral bracts. As this occurs, gaps begin to appear in the top of the pappus, down which the top of the florets are visible (e.g. Plate 23D). When the involucral bracts are approximately vertical, the filiform florets begin to open. The filiform florets appear to open in quick succession, with only a slight delay between the first and the last to open (Plate 23D). After the filiform florets reach anthesis, the corolla and the style continue to elongate, so that the corolla is presented above the involucral bracts, and the junction of the style arms is well clear of the pappus. The tubular

florets begin to open when the top of the florets is above the pappus. By this stage the styles of the filiform florets are well extended. The initial group of tubular florets usually contains one or two florets. The next group of tubular florets begins to present pollen when the previous group is just entering the female phase. Subsequent groups normally contain two or three tubular florets, although the last few florets usually open singly (Plate 23E, F). Occasionally the styles of the filiform and outer tubular florets begin to brown while the last tubular floret is still presenting pollen. However, in most capitula all the florets will be open and presenting pollen before the styles begin to brown. The outer tubular florets usually brown first, followed by the filiform then the inner tubular florets. Occasionally the filiform florets are the last to brown, although it is more usual for the central tubular florets to brown last.

Raoulia subsericea (F: 15.8, T: 17.8)

The involucral bracts initially form a tightly closed bud. The involucral bracts gradually begin to separate, angling outwards. The involucral bracts continue to angle outwards, so that by the time the filiform styles are taller than the pappus, the involucral bracts are angled outward at 45° to 50°. When fully open the involucral bracts spread to approximately 90°. When the involucral bracts are just past upright, the filiform florets begin to open. Initially the filiform florets are only just visible down the side of the pappus, however the styles of the filiform florets elongate during anthesis, so that they quickly become taller than the pappus. When the involucral bracts are spread to approximately 45°, the top of the first tubular florets are usually visible (e.g. Plate 22E, floret in top right corner). Usually the initial group of tubular florets contains 1 to 3 florets, but occasionally it may contain up to six florets. The tubular florets begin to open when the top of the corolla is above the pappus tips, with subsequent groups opening as the previous group of tubular florets is just completing pollen presentation (Plate 22D), or entering female phase. The last tubular floret is generally presented singly. By this stage any filiform florets which have not browned, are presented above the level of the pappus and are usually well curled (Plate 22F). The first filiform floret usually begins to brown when approximately two thirds of the tubular florets are open. Occasionally, however, the first floret will not brown until all the tubular florets are open. The first of the tubular florets usually begins to brown at about the same time as the filiform florets (Plate 22F). The styles of both types of floret usually brown before the corollas.

Raoulia tenuicaulis (F: 5.5, T: 3.8)

The involucral bracts initially form a tightly closed bud. The involucral bracts do not appear to spread as the capitulum develops, instead the involucral bracts appear to be forced apart by the developing florets. The filiform florets all appear to reach anthesis at the same time, first becoming visible as pairs of style arms extending through a very small opening between the tip of the involucral bracts. The filiform styles continue to elongate, and the style arms begin to spread horizontally. When the filiform styles are well extended, but before the junction of the style arms becomes visible, the top of the initial tubular floret(s) becomes visible between the closely pressed bract tips. The initial tubular floret usually appears singly, or occasionally as a pair. When the top of the tubular floret is well above the top of the involucral bracts, the corolla splits, and the floret begins to present pollen. When two florets appear together, there is a slight delay between the first and second tubular florets begin to present pollen. The next group of tubular floret becomes visible when the previous group is just entering the female phase, with each of the subsequent florets opening singly. By the time the second group of tubular floret opens, the styles of the filiform florets are fully extended, and usually extensively curled. As the tubular florets reach mid to late female phase the corolla begins to collapse. The tubular corollas are the first floral part to brown, usually followed by the first tubular and the filiform florets. However, a filiform floret will occasionally still be presenting its style after all the other florets are brown.

Glasshouse Observations

The flowering patterns of the capitula under glasshouse conditions varied only slightly within a species, so that a common pattern was readily apparent for each species. These common patterns are presented in Figure 3.30 to Figure 3.32. For some species, however, (e.g. *Raoulia hookeri*) more than one pattern was observed, and in these species all of these patterns are depicted.

The capitulum pattern for each species is represented by three rows (Figure 3.30 to Figure 3.32). The bottom row indicates the proportion of filiform florets presenting their styles, the middle row indicates the proportion of tubular florets presenting pollen, and the top row the proportion of tubular florets presenting their style. In some patterns a row is blank

(e.g. *Gnaphalium audax*). This indicates that this phase was not observed. However, in *Ozothamnus leptophyllus* the bottom row is blank because no filiform florets are present in the capitula of this species.

Three features are common to all the capitula patterns. Firstly, with the exception of *Raoulia haastii* (Figure 3.31), the tubular florets do not present pollen before the filiform florets have begun to present their styles. In *R. haastii*, the first tubular floret was observed to present pollen before the filiform florets reached anthesis, and was generally in the female phase when the filiform florets opened.

The second common feature is the narrow width of the middle row, i.e. the proportion of tubular florets presenting pollen. In nearly all of the species observed, the proportion of tubular florets presenting pollen at any one time is small. The most notable exception to this is the second pattern of *Raoulia australis* (Figure 3.31), in which the proportion of tubular florets presenting pollen at one time is comparatively high. In all species pollen presentation by each tubular floret occurred in only the first or first and second day of anthesis, even though the pollen was not removed from the florets.

The third common feature of the capitula patterns is the rapid emergence of the filiform florets, and the high proportion of these which remain open at one time.

There are also several differences amongst the species. Most prominent of these is the difference in the length of time the capitulum is at anthesis. For example, the length of anthesis of a single capitulum is nearly 40 days in *Helichrysum bellidioides, H. filicaule, Leucogenes grandiceps* (Figure 3.31) and *Raoulia grandiflora* (Figure 3.32). In contrast, the capitula of *Gnaphalium traversii, G. audax* (Figure 3.30), and *R. australis* are only open for approximately 14 days (Figure 3.31).

The species also differ slightly in the timing of the three components. For example, *Helichrysum intermedium* (Figure 3.31) is the only species observed to continue presenting pollen once the filiform florets are no longer at anthesis. Another example is the length of the female phase of the tubular florets. In nine species style presentation in the tubular florets ends later than style presentation by the filiform florets (e.g. *R. grandiflora*), in three species (*R. haastii, R. hookeri*, and *R. subsericea* (Figure 3.31, Figure 3.32)) the tubular

and filiform florets end anthesis at the same time, while in the other three species (*G. audax*, *G. traversii*, and *H. bellidioides* (Figure 3.30)) the filiform florets end anthesis later than style presentation by the tubular florets.

In the species that were observed to have two general capitula patterns, the variation occurs in the timing of the components. This variation occurs in three main ways: (1) the timing of the start of pollen presentation with respect to anthesis of the filiform florets (e.g. *R. hookeri*, *R. subsericea*, *R. tenuicaulis*, *G. traversii*); (2) the presentation (or lack of presentation) of the style in tubular florets (e.g. *G. audax,* Figure 3.30); and (3) the distribution of the peaks in pollen presentation (e.g. *R. haastii*, *G. audax*).

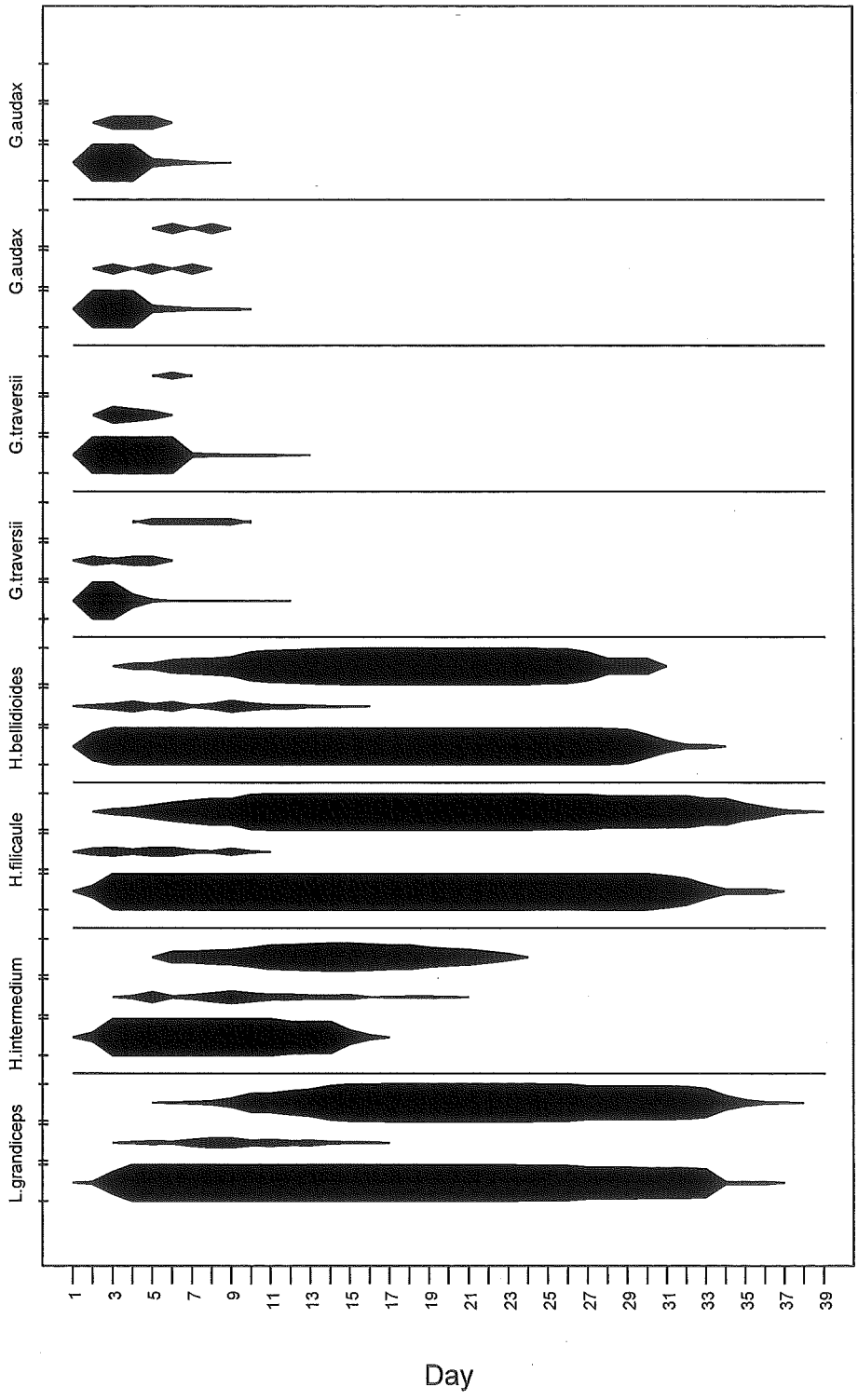


Figure 3.30: The general pattern(s) of capitulum phenology of *Gnaphalium audax, G. traversii, Helichrysum bellidioides, H. filicaule, H. intermedium,* and *Leucogenes grandiceps* observed in the glasshouse. For each species the rows represent the proportion of filiform florets at anthesis (bottom row), and the proportion of tubular florets presenting their pollen (middle row) or style (top row). (Maximum bar widths indicated on the left axis.)

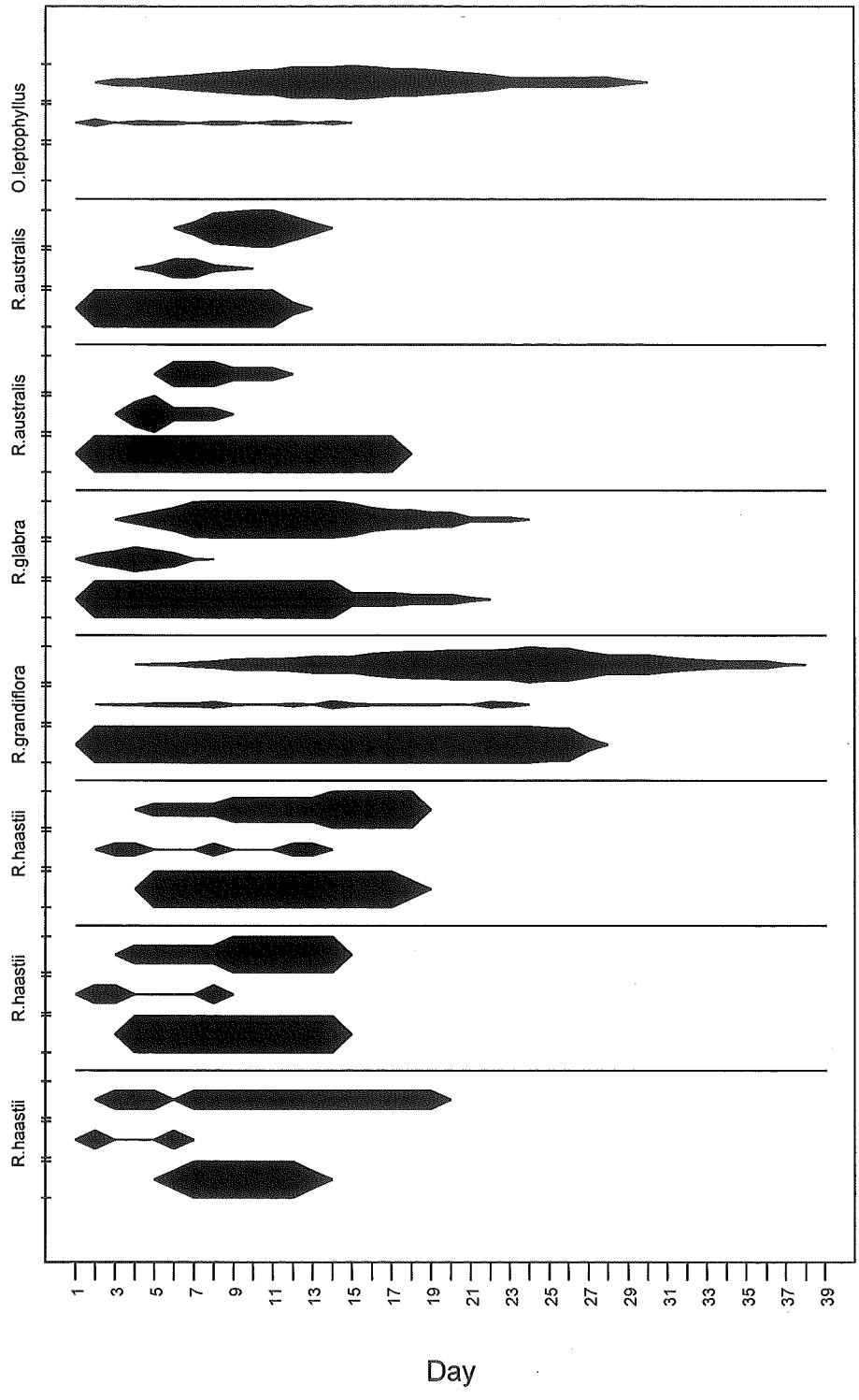


Figure 3.31: The general pattern(s) of capitulum phenology of *Ozothamnus leptophyllus, Raoulia australis, R. glabra, R. grandiflora,* and *R. haastii* observed in the glasshouse. For each species the rows represent the proportion of filiform florets at anthesis (bottom row), and the proportion of tubular florets presenting their pollen (middle row) or style (top row). (Maximum bar widths indicated on the left axis.)

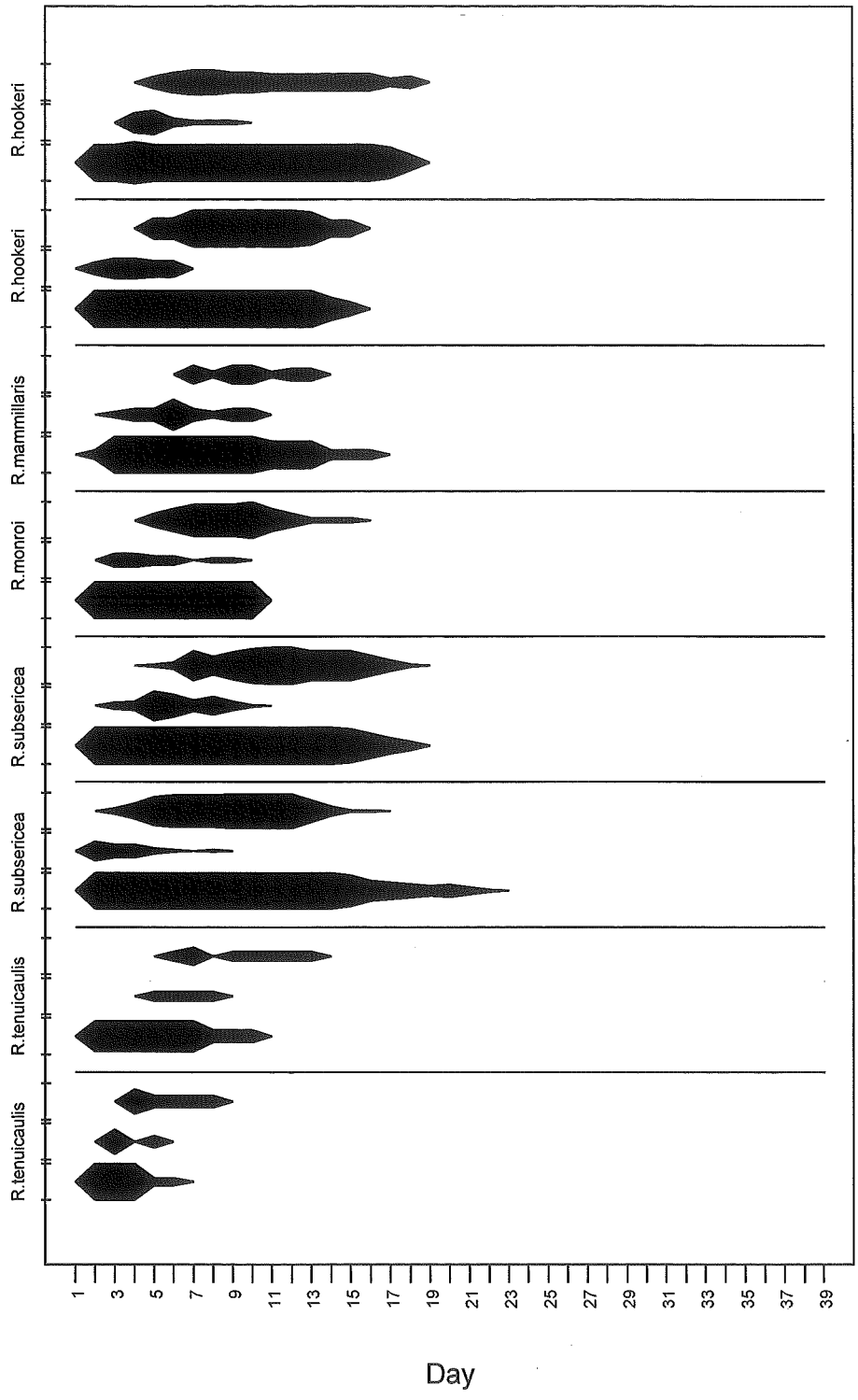


Figure 3.32: The general pattern(s) of capitulum phenology of *Raoulia hookeri, R. mammillaris, R. monroi, R. subsericea,* and *R. tenuicaulis* observed in the glasshouse. For each species the rows represent the proportion of filiform florets at anthesis (bottom row), and the proportion of tubular florets presenting their pollen (middle row) or style (top row). (Maximum bar widths indicated on the left axis.)

3.4.5 Floret phenology

For each species the phenological stages of the filiform and tubular florets are described below. For each species, except *G. traversii*, a figure with illustrations is provided. These are indicated after the species name, but for brevity the stages depicted in these diagrams will be referred to without further reference to the figure number. Stages of the filiform phenology are indicated by an "F" proceeding a number, and the tubular floret stages by an "T".

The terms used in these descriptions are the same as used in the descriptions of the capitula phenology.

Gnaphalium audax (Figure 3.33)

Filiform: The styles of the filiform florets are first visible level with, or just below, the top of the involucral bracts. The styles are initially very short (F1), however the style arms are separated as soon as they are visible. As anthesis continues the style arms elongate and divide further (F2, F3). The style occasionally browns before the style arms are fully extended, however, if this does not occur the style arms continue to extend and at the same time begin to curl (F4). As anthesis continues the floret appears to be pushed upward by the developing achene, so that by the end of anthesis the corolla, which is not initially visible, is minutely visible just level with the top of the involucral bracts. The style first begins to brown in the arms, and gradually begins to withdraw (F5). By the time the style is totally brown, the style arms will have withdrawn so the style arms are just above the level (F6), or even inside, the corolla (F7).

Tubular: When first visible, the corollas of the tubular florets have already split. The tubular florets develop quickly, so that soon after they become visible the stamen tube is visible, and begins presenting pollen. The stamen tube only extends a short distance above the corolla tube when presenting pollen (T4). During anthesis the corolla lobes of the tubular floret are only just above the level of the pappus. If space permits, the corolla lobes will spread so that they are presented horizontally. Once pollen presentation is complete, the stamen tube withdraws rapidly to the level of the top of the corolla tube, and the style is presented (T5). Once exposed the style arms rapidly separate (T6). At the end of the

female phase the style withdraws into the corolla tube (T7) and the corolla begins to brown (T8).

Gnaphalium traversii

The stages of the filiform and tubular floret phenologies are the same as those of *G. audax*.

Helichrysum bellidioides (Figure 3.34)

Filiform: The filiform florets are only just visible prior to opening, being placed well down the side of the capitulum, amongst the pappus (F1). The filiform florets open when still well below the top of the pappus hairs. Initially the style arms are only very slightly separated (F2), spreading radially (Plate 20B, C). As the florets age, the style elongates and the style arms spread, so that the junction of the style arms is well clear of the top of the corolla tube (F4, F5). By this stage the corolla of the outermost filiform florets is visible, however the corolla of the inner filiform florets remains hidden by the pappus throughout anthesis. As the filiform florets reach the end of anthesis, the styles begin to withdraw slowly back into the corolla tube (F6). Browning begins at the tip of the style arms (F7), and gradually progresses down the style. When totally brown, the style has withdrawn into the corolla so that the junction between the style arms is no longer visible (F8). The corolla browns after the style (F9).

Tubular: The tops of the tubular florets become visible shortly before they open (T2). The corolla splits when the top of the floret is level with, or sometimes just above, the pappus tips (T3). Once the corolla has split the stamen tube rapidly elongates, and, when approximately 1 mm above the corolla, begins presenting pollen (T4). When the floret begins to present pollen, the corolla lobes are slightly curved outwards, and the corolla is at its most prominent. When all the pollen has been presented, the style is visible at the top of the stamen tube. The style then extends beyond the top of the stamen tube (T5), which allows the stamen tube to withdraw back into the corolla (T6). The stamen tube withdraws to, or below, the level of the corolla lobes, by the time the style arms are fully spread (T7). As the female phase continues, the corolla retracts to below the level of the pappus hairs (T7, T8). Toward the end of the female phase, the style begins to withdraw into the corolla tube (T9), and then begins to brown, starting along the sides of the style arms (T10). The style continues to brown and withdraw, so that when the style is completely brown only a

small part of the style arms will be above the top of the corolla tube (T11). The corolla usually starts to brown when the style is almost completely brown.

Helichrysum filicaule (Figure 3.35)

Filiform: The filiform florets normally become visible between the involucral bracts and pappus while the corolla is still closed (F1, F2). Soon after they first become visible, and while still well below the top of the pappus, the top of the corolla splits. Once the corolla has opened the corolla lobes spread rapidly. During the early part of anthesis the corolla continues to extend, so that by the time the junction of the style arms is well clear of the corolla tube, the top of the corolla tube is approximately level with the involucral bracts. As soon as the corolla splits, the style is visible just below the top of the corolla tube, with the style arms slightly separated. The style arms then begin to gradually elongate (F3, F4). As the style elongates, the style arms spread and gradually begin to curl (F5). The style may brown very early in anthesis; if this occurs the style does not extend further, and usually withdraws into the corolla slightly. If the style does not brown early in anthesis, it continues to elongate until the junction of the style arms is well above the top of the pappus, and the style arms continue to curl (F6). The style normally begins to brown before the corolla; however, if the floret is open for a long period, the corolla may begin to brown first. The style usually begins to brown in the arms first (F7), gradually withdrawing into the corolla tube as it does so. When completely brown, the style is withdrawn so that the junction of the style arms is either level with (F9), or below (F8), the top of the corolla tube. The corolla has usually started to brown before the style is completely brown. The corolla begins browning at the tips of the lobes first, and gradually collapses inward as it browns.

Tubular: The tubular florets are visible from above, through gaps in the pappus while still young (T1). As the tubular florets develop they become taller, and gradually swell at the top (T2). When approximately level with the top of the involucral bracts, and just below the level of the pappus tips, the corolla begins to split at the top. As soon as the corolla lobes have spread sufficiently the stamen tube begins to elongate (T3). While the stamen tube is extending, the corolla lobes continue to separate, so that by the time the stamen tube is fully extended and beginning to present pollen, the corolla lobes are fully spread and the corolla lobes have usually begun to curl (T4). During pollen presentation, and the early part of the female phase, the corolla forms a prominent cup. When it first emerges and

during presenting pollen, the stamen tube is marked by purple colouration near the tip and on the ridges down its length (T4). At the end of pollen presentation, the style is visible just below the top of the stamen tube. The styles then begin to separate inside the stamen tube. This appears to force the stamen tube to start splitting (T5), and subsequently to withdraw a short distance into the corolla. Once free of the stamen tube, the style continues to extend. As the style lengthens, the style arms continue to separate and begin to curl. When the style is fully extended, the junction between the style arms is approximately level with the top of the now discoloured stamen tube (T6). If the style does not begin to brown at this stage, the style arms continue to curl and may eventually almost touch the style below the junction of the arms. Browning of the floral parts is variable, with two general patterns observed. In the first pattern, the style will brown first, normally starting on the side of the style arms, approximately half way along the style arm. The style arms then brown in both directions, with the style withdrawing into the corolla as this occurs. When the style is brown, the corolla begins to brown and becomes squashed (T7). In the second pattern, the corolla and style each brown in the same manner, except that the corolla begins browning before the style. In both patterns the style withdraws to varying extents; from only withdrawing a very small distance, to withdrawing so the junction between the style arms is no longer visible. The tubular florets gradually bend to varying extents during anthesis. The outermost florets bend to the greatest extent (e.g. T5), while the central tubular florets do not bend at all (e.g. T7).

Helichrysum depressum (Figure 3.36)

Tubular: The top of the floret becomes visible while still well below the level of the pappus (T1). As each floret develops it gradually becomes taller, and when level with the top of the pappus, the corolla begins to split. As soon as the corolla lobes have separated sufficiently, the stamen tube begins to elongate (T2). As the stamen tube is elongating, the corolla lobes continue to separate, so that by the time pollen presentation begins, the corolla lobes are fully spread, and usually curve outwards just above the top of the pappus (T3). As anthesis continues the corolla lobes continue to curve, and if space permits, will curl under at the tips. The corolla is most prominent during pollen presentation, forming a narrow flute at the bottom of which nectar is sometimes visible. When the stamen tube is fully extended, the floret begins presenting pollen. At the end of pollen presentation, the style is visible at the end of the stamen tube, with the tightly closed style arms just above the top of the stamen tube. The stamen tube then begins to withdraw into the corolla and

splits down one side. When fully withdrawn, the top of the stamen tube is usually below the level of the corolla lobes. When the stamen tube has withdrawn approximately half way into the corolla, the style arms begin to spread rapidly (T4). The style is at its greatest extension, just after the style arms have split. It then withdraws slowly back into the corolla as anthesis continues. As the style arms spread they gradually curve (T6), and may eventually curl (T8), if they do not brown first. As the floret enters female phase, the corolla becomes squashed by the later opening florets. Additionally, when the corolla begins to brown it withdraws into the pappus slightly. Browning of the style and corolla is variable. In most florets the style browns before the corolla (T9), withdrawing further into the corolla in the process (T10, T11). Occasionally, however, the corolla begins to brown before the style. In both patterns, when totally brown, the style only just extends out of the corolla tube (Plate 22 C, T11).

Helichrysum intermedium (Figure 3.37)

Filiform: The filiform florets are visible between the pappus hairs while still well below the top of the pappus. The florets gradually extend (F1), and when level with, or just below, the top of the pappus, the corolla tube begins to split (F2). The corolla lobes continue to split and separate, and begin to curl during the early part of anthesis. Toward the end of anthesis, the corolla lobes may have curled sufficiently to touch the corolla tube (F5). A prominent feature of the filiform anthesis is the bending of the floret. This process begins almost immediately following the splitting of the corolla, and continues throughout anthesis, so that by the end of anthesis the floret will usually be bent to 90° or more. As soon as the corolla has split sufficiently, the style begins to lengthen (F3). Initially the style arms are held close together. However, as the style lengthens, the style arms separate and curve outwards (F4). When the style arms are fully spread, they are held approximately horizontal and are slightly curved with the tips usually pointing towards the corolla tube (F5). As the style browns, it withdraws into the corolla (F6) so that when completely brown it protrudes only just above the top of the corolla. The browning of the floret occurs in one of two patterns. First, the corolla may brown completely before the style begins to brown. Or, second, the style browns earlier than the corolla, and is almost completely brown before the corolla starts to brown. The style usually begins to brown at the tip of the style arms. The corolla usually begins browning in the lobes (F7).

Tubular: The tubular florets become visible while still well below the level of the pappus (T1). Each floret gradually extends and the corolla swells slightly towards the top (T2). When the top of the floret is above the level of the pappus, the corolla begins to split. Initially this process appears to be slow (T3), but appears to speed up once the stamen tube is free of the corolla (T4). Once the stamen tube is free of the corolla it extends rapidly, and begins to present pollen. By this stage the corolla lobes are fully extended, and are usually slightly curled (T5). As pollen presentation continues, the corolla lobes continue to curl, so that as the floret enters female phase, the corolla lobes are curled under, often touching the sides of the corolla tube. When pollen presentation is complete, the style appears to be pushed through the top of the stamen tube. Once this has occurred, the stamen tube begins to withdraw back in to the corolla (T6), and the style arms begin to separate. As the style arms separate, they gradually curl (T7, T8), so that toward the end of anthesis they may have curled sufficiently to be touching the top of the corolla (T9). Toward the end of anthesis, the style begins to gradually retract back into the corolla tube. The floret begins to brown in either the corolla, or the style, with either structure often nearly completely brown before the other structure also begins to brown. A prominent feature of the outer tubular florets is the bending of the florets in the same manner as the filiform florets.

This is most prominent in the outer tubular florets, and gradually becomes less prominent in the florets as they are placed closer to the centre of the capitulum.

Leucogenes grandiceps (Figure 3.38)

Filiform: The filiform florets usually first become visible as a pair of style arms emerging from between the pappus and the involucral bracts. However, in some capitula, the top of the florets are visible before the floret opens (F1). The short corolla lobes of the filiform floret vary from being not spread at all, to being well spread. The corolla is only minutely visible during anthesis. As soon as the corolla lobes split (F2), the style begins to rapidly elongate. The style arms begin to separate as soon as they emerge (F3), so that by the time they are taller than the pappus the style arms are well spread (between F4 and F5). The style continues to elongate throughout anthesis, to a maximum point at which the junction of the style arms is above the top of the pappus (F5). At full anthesis, the style arms are presented just above the pappus tips, and are slightly curled. At the end of anthesis the style begins to brown at the tip of the style arms first, and the style gradually retracts back into the corolla tube. When completely brown, the style arms only protrude a short

distance out of the corolla (F6). The corolla usually browns after the style has already browned.

Tubular: The tubular florets are first visible when still well below the level of the pappus (T1). The florets gradually elongate (T2), and when above the level of the top of the pappus, begin to split. Once the corolla has split sufficiently the stamen tube begins to elongate. The corolla lobes continue to separate, so that they are fully divided and partially curled back by the time pollen presentation begins (T3, T4). The corolla is most prominent during pollen presentation. The corolla of the tubular florets tends to be squashed by other, later opening tubular florets, so that by late female phase the corolla tube is almost squashed flat (T9). When pollen presentation ends, the style is level with the end of the stamen tube. The style appears to continue to elongate, and once free of the stamen tube, the style arms begin to separate (T6). Once the top of the style is free of the stamen tube, the stamen tube begins to withdraw gradually into the corolla tube, and to split down one side. When the style is first presented it is held well above the level of the pappus and corolla (T7, T8), however as the floret ages, the style gradually retracts into the corolla. When totally brown the style will be below the level of the corolla lobes (T10, T11). The style begins browning at the tip of the style arms first. The corolla usually begins to brown after the style is completely brown.

Ozothamnus leptophyllus (Figure 3.39)

Tubular: The top of the corolla becomes visible while still well below the level of the pappus (T1). At this stage there is often a red tinge to the top of the corolla. As the floret develops, it extends and the top portion of the corolla swells (T2, T3). By the time the top of the floret is above the top of the pappus, the top part of the corolla is extremely swollen (Plate 24B, T4). This swollen end of the floret is usually above the top of the pappus before the corolla begins to split from the top (T5). Once started, the corolla splits rapidly (T6), allowing the stamen tube to extend (T7). As the corolla splits, the lobes start to curve outwards, so that by the time the floret is presenting pollen, the corolla lobes are fully extended, and usually curl under at the tips (T7). By the end of anthesis the corolla lobes are often curled extensively, so that they may touch the side of the corolla tube. The corolla has often been squashed by later opening florets by the time the floret reaches the middle of the female phase. Once pollen presentation is complete, the style is level with, or just below, the top of the stamen tube. When the style is clear of the stamen tube, the

stamen tube begins to withdraw and splits down one side (T8). By the time the stamen tube has withdrawn to the level of the corolla tube, the style arms are well separated, and slightly curled (T10). At this point the style arms are presented well above the top of the corolla. As the female phase continues, the style arms continue to curl so that they point back in towards the style (T11). Towards the end of the female phase, the style begins to withdraw into the corolla tube, and begins to brown approximately mid way down the style arms (T12). The style continues to brown, and withdraws into the corolla tube, which itself has often started to retract slightly. When completely brown the style usually withdraws so that only approximately half the length of the style arms are visible (T14). The corolla begins browning after the style is completely brown. When completely brown, the corolla is usually level with, or just below, the top of the pappus hairs.

Raoulia australis (Figure 3.40)

Filiform: The corolla lobes of the filiform florets separate before the florets are visible (F1). However, as anthesis continues, the corolla continues to extend, and once the corolla is clear of the involucral bracts, the corolla lobes spread slightly (F3). Before the corolla becomes visible the style then appears to lengthen, and is initially presented pressed between the pappus and the involucral bracts (F2). The style also continues to extend, so that the style arms are presented horizontally, just above the top of the involucral bracts and pappus (F4). As the style arms extend, they curl extensively, eventually curling back to touch the corolla (F5). The style arms begin to brown at the tips, and are usually totally brown before the corolla begins to brown (F6). When the corolla begins to brown the floret appears to withdraw slightly, but remains visible.

Tubular: The swollen end of the tubular florets usually only become visible when they are just below, or level with, the top of the pappus hairs (T2). Once the top of the corolla is just above the top of the pappus, the corolla lobes begin to separate (T3). Throughout anthesis the corolla lobes do not spread widely, usually opening just sufficiently to allow the stamen tube to emerge (T4). Once the corolla has split, the stamen tube extends a small distance above the corolla lobes and begins to present pollen (T5). At the end of pollen presentation the stamen tube begins to withdraw back into the corolla tube. At this point the style becomes visible, usually being placed well below the top of the stamen tube, or occasionally just below the top of the tube. The stamen tube then appears to split down more than one side, allowing the style arms to separate very slightly (T6). However, in

some tubular florets the style arms do not appear to separate at any stage of anthesis. In nearly all tubular florets (>95% of those examined) the style does not extend above the top of the corolla tube. In the remaining few per cent of florets, the style is presented just above the corolla lobes, and the style arms separate sufficiently only to create a narrow V-shape. Both the style and stamen tube continue to retract, so that by the time the floret begins to brown, they are below the level of the corolla (T7). The corolla normally begins browning before the style, occasionally, however, the style will brown first.

Raoulia glabra (Figure 3.41)

Filiform: The filiform florets become visible well below the level of the pappus, pressed between the pappus and the involucral bracts (F1). The corolla splits while still well below the level of the pappus, and the style is immediately visible. The corolla continues to elongate and at its maximum extension is presented just below, or level with, the top of the pappus. At mid anthesis the corolla lobes may spread, forming a very narrow cup. The style arms separate before they are taller than the corolla tube, and continue to spread as the style rapidly elongates (F2). By the time the style arms are spread horizontally the junction of the style arms is well above the top of the corolla tube (F3). As the floret ages the style continues to elongate and the style arms begin to curl under (F4). When fully extended the style arms are held well above the pappus tips, and the style arms have curled so that they point back towards the style (F5). The style usually begins to brown first in one style arm (F6). As the style browns (F7) it withdraws very slightly into the corolla, but still extends well above the corolla tube when completely brown. The corolla browns after the style.

Tubular: The top of the tubular floret is first visible while still well below the level of the pappus (T1). As the floret develops, it gradually extends and the top portion of the floret swells (T2). When approximately half of the swollen portion is visible above the pappus, the corolla begins to split from the top. By the middle of the male phase the corolla lobes are well spread, and the corolla forms a distinct cup (Plate 19F, T4). During anthesis the corolla lobes of the tubular florets curve over (e.g. T11), and if space permits will eventually curl under (e.g. T8). As soon as the corolla has split sufficiently the stamen tube rapidly elongates (T3). The florets often begin to present pollen before the corolla lobes have spread beyond the stage pictured in T3. When all the pollen has been dispersed, the top of the style is visible above the level of the stamen tube (T5). At the start of the female phase, the stamen tube withdraws very slightly into the corolla, but remains

extended beyond the top of the corolla. However, the stamen tube splits down one side, and usually falls away from the style (T9). As the floret enters the female phase, the style continues to elongate, and the style arms begin to spread and curve (T6, T7, T8). By late female phase the style arms have usually curled sufficiently so that they almost touch the style (T10, T11). At the end of anthesis the style begins to brown in the arms (T12), and the style withdraws slightly into the corolla tube (T13). When completely brown the style is still extended well above the corolla. The corolla may brown before, with, or after the style, with individual tubular florets within the same capitulum showing different patterns.

Raoulia grandiflora (Figure 3.42)

Filiform: The filiform floret first becomes visible as a pair of style arms between the outer pappus, close to the involucral bracts. By this stage, the top of the style is well clear of the corolla tube. Initially the style arms are closely pressed together (F1), however, as the style continues to extend the style arms separate rapidly (F2). By the time the style arms are level with the top of the pappus they are fully spread (Plate 26E). If the style does not brown shortly after reaching this stage, it continues to elongate, so that the style arms are eventually presented well above the level of the pappus (F3). The style usually begins browning in one style arm first. Once the style has started to brown it retracts back into the corolla, so that by the time it has fully browned, the style is well below the level of the pappus tips (F4). The corolla appears to brown shortly after opening, and is not visible during anthesis.

Tubular: The tubular florets become visible between the pappus shortly before they open (T1). As each floret extends the top of the corolla swells (T2). When the top of the floret is approximately level with the pappus tips, the corolla begins to split rapidly. The corolla lobes generally curve outward slightly, and remain approximately level with the pappus tips throughout anthesis. Following pollen presentation the corolla tube is gradually squashed, so that by the end of anthesis the sides of the corolla tube are squashed together. Once the corolla lobes have separated sufficiently the stamen tube elongates rapidly. By the time the stamen tube is fully extended, and beginning to present pollen, the corolla is fully open, and is at its most prominent (T3). When all the pollen has been dispersed, the style is visible level with, or just above, the top of the stamen tube. Once pollen presentation has ended, the stamen tube rapidly withdraws into the corolla (T4). By the time the stamen tube is level with the top of the corolla the style arms are slightly spread

(T5). As the female phase continues the style gradually withdraws back into the corolla, so that by the time the style arms begin to brown they are held just above the level of the corolla (T6). The corolla browns after the style has already browned.

Raoulia haastii (Figure 3.43)

Filiform: The first part of the filiform floret to become visible is the style arms, which are pressed between the open tubular floret and the involucral bracts (F2). The style arms continue to elongate, and by the time they have begun to bend outwards the corolla becomes visible (F3). The style continues to extend, and the style arms begin to curl (F4, F5, F6), so that by late anthesis the style arms have often curled back onto themselves (F7). Towards the end of anthesis the style begins to slowly withdraw into the corolla, so that when completely brown it only just extends out the top of the corolla (F8). If the corolla extends sufficiently to clear the involucral bracts, the corolla lobes spread apart widely (Plate 27C, top left capitulum). The corolla browns after the style, withdrawing below the level of the involucral bracts when completely brown.

Tubular: The tubular florets extend, and the top of the corollas swell slightly before they become visible (T1, T2). When the top of the corolla is above the involucral bracts, the top of the floret swells further, and begins to split from the top. When the corolla lobes have separated sufficiently, the stamen tube begins to elongate (T3). By the time pollen presentation begins, the corolla lobes are usually fully spread, and usually curl down at the tips (T4). However, as the initial tubular florets enter female phase the corolla tends to withdraw into the involucral bracts slightly, forcing the corolla lobes to become upright again (T5). This does not usually occur in the later opening tubular florets until the end of anthesis. At the end of pollen presentation, the style is usually extended just above the end of the stamen tube, so that the last of the pollen is usually presented on the ends of the style. As the style arms begin to separate, the stamen tube gradually withdraws into the corolla tube, so that when withdrawn to the full extent, the top of the stamen tube is just above the level of the corolla (T6). Occasionally the stamen tube will split down one side. As female phase continues, the style arms continue to extend and separate, curving over as they extend (T6). The end of anthesis is indicated by the browning of the style. This usually occurs before the corolla browns; however, occasionally the corolla will be brown well before the style starts to brown. As the style browns it gradually retracts in the corolla

tube. At the end of anthesis the corolla and other floral parts will have withdrawn below the top of the involucral bracts.

Raoulia hookeri (Figure 3.44)

Filiform: The filiform floret extends (F1, F2) and opens just as it becomes visible from above (F3). The corolla appears to extend during the early part of anthesis, so that in some florets it becomes visible just above, or level with, the top of the pappus and involucral bracts. After the corolla splits the style begins to elongate, with the style arms spreading and curling as they extend (F4, F5). By the time the corolla has reached its maximum height, the style arms are usually well extended, and slightly curled (F5). The junction of the style arms is not visible at any stage of anthesis, so that the style arms are presented close to the top of the pappus. By the end of anthesis, the style arms are usually extensively curled (F6). Once the style begins to brown it gradually withdraws into the corolla (F7), so that by the time it is totally brown, only a small length of the style arms is visible (F8). The corolla browns after the style is brown.

Tubular: The top of the tubular florets become visible while still well below the level of the pappus (T1). As each floret develops, the top part of the floret swells slightly (T2). When the top of the floret is well above the top of the pappus, the corolla splits, and the stamen tube rapidly elongates (T3). The corolla lobes do not usually spread past the vertical, so that by the time pollen presentation begins, they are fully spread (T4). During the "female" phase of anthesis the corolla tube of the tubular floret is usually squashed by the later opening florets. At the end of pollen presentation, the stamen tube quickly retracts back into the corolla, so that it is level with, or only just above, the top of the corolla lobes (T5). As a result of this process, the style becomes exposed. When first visible the style is at its maximum height. In some florets (less than 50% of those examined) the style arms separate, but do not spread very widely (T6). Soon after the style becomes visible it begins to withdraw into the corolla, and is usually below the top of the corolla tube before it begins to brown (T7). Once the style is below the top of the corolla, the corolla begins to brown. When the corolla is brown, the style arms begin to brown (T8).

Raoulia mammillaris (Figure 3.45)

Filiform: The filiform floret becomes visible well down the side of the pappus (F1). Soon after it becomes visible the top of the corolla opens (F2), and the style begins to elongate.

The corolla continues to extend, until it reaches a maximum just below the top of the pappus. During anthesis the corolla lobes spread, and are visible through gaps between the involucral bracts, or from above. The style continues to elongate once the corolla has split, and the style arms spread and gradually curve (F3, F4, F5). At peak anthesis, the style has elongated so that the junction of the style arms is level with, or just above, the top of the corolla tube (F6). At this point the style arms are well spread, and held above the level of the pappus. At the end of anthesis, the style begins to brown from the tip of the arms (F7), and gradually withdraws back into the corolla tube (F8). When completely brown approximately half of the style arms protrude from the corolla tube (F9). The corolla begins to brown after the style is brown, and often becomes squashed late in anthesis.

Tubular: The tubular floret becomes visible between the pappus, while still well below the top of the pappus (T1). The floret continues to elongate, and when the top is just above the level of the pappus the corolla splits from the top (T2). Once the corolla has split sufficiently, the stamen tube begins to elongate. The corolla lobes are usually fully spread, and the tips have started to curl by the time the stamen tube is at half its full extension. At this stage the corolla forms a deep cup (T3). When the stamen tube reaches its full height, pollen presentation begins. During pollen presentation the top of the stamen tube is marked by a purple colouration. Once all the pollen has dispersed, the style is visible level with the top of the stamen tube. The stamen tube then begins to retract (T4), allowing the style arms to spread slightly (T5). As the stamen tube retracts into the corolla, it often splits down one side, and may fall away from the style. Following pollen presentation, the corolla gradually becomes squashed, so that by the end of the female phase the corolla tube may be pressed against the style. The styles begin to withdraw into the corolla before the female phase is over, so that the junction of the style arms is usually level with the top of the corolla when the style begins to brown. The tubular florets always appear to brown in the corolla first. Once the corolla is totally brown, the style begins to brown along the sides of the style arms (T6). When totally brown, approximately one third of the style arms remain extended above the corolla tube (T7).

Raoulia monroi (Figure 3.46)

Filiform: The top of corolla becomes visible while still below the level of the pappus (F1). Soon after it is visible, the top of the corolla splits, and the style begins to elongate (F2). Both the style and the corolla appear to extend throughout anthesis. The corolla usually

reaches a maximum height just above the level of the involucral bracts, at which point the corolla lobes is fully spread. As the style elongates, the style arms spread, and slowly begin to curl (F3, F4). When fully extended, the junction between the style arms is well clear of the top of the corolla tube (F4), and is presented above the level of the pappus. The style usually begins browning in the arms first, and slowly withdraws into the corolla during this process (F5). When completely brown the style still extends out of the corolla. The corolla usually browns after the style.

Tubular: The tubular floret becomes visible while still well below the level of the pappus (T1). As the florets extend, the top portion of the corolla gradually swells (T2). When the top of the corolla is level with the pappus, the corolla begins to split. Once the corolla has split sufficiently the stamen tube begins to elongate rapidly (T3). By the time pollen presentation begins, the corolla lobes are fully spread, and usually slightly curled at the tips (T4). The corolla is at its most prominent during the male phase, and during female phase is gradually squashed by later opening florets. During pollen presentation, the top of the stamen tube is coloured by a purple band just below the tops of the stamen. This band fades once the floret is in female phase. At the end of pollen presentation, the style is visible at the end of the stamen tube. Once the top of the style extends out of the top of the stamen tube, the style arms begin to spread. This appears to split the stamen tube, which also withdraws into the corolla tube slightly (T5). As the style arms spread, they gradually curl, so that they eventually point back toward the style (T6). The style begins to brown after the corolla has started to brown, and usually browns along the sides of the style arms first. The style appears to withdraw into the corolla tube slightly when completely brown.

Raoulia subsericea (Figure 3.47)

Filiform: The top of the filiform floret becomes visible through the pappus, just before it opens (F1). Upon opening, the tips of the style are pressed tightly together. As the style elongates the style arms begin to separate from the tip (F2). The style arms are initially held closely spaced (F3), and only curve at the tips (F4). As anthesis progresses, the style extends so that the junction of the style arms is well clear of the top of the corolla tube. By this stage the style arms are above, or level with, the pappus, and the arms have started to curl (F5). When the floret is at full anthesis, the corolla is sometimes visible between the pappus hairs. The filiform floret begins browning at the tips of the style arms (F6, F7). As

the style browns, it withdraws into the corolla so that when fully brown the full length of the style arms is not visible. The corolla browns after the style.

Tubular: The top of the tubular floret becomes visible shortly before it opens (T1). When the top of the tubular floret is approximately level with the pappus, the corolla begins to split from the top, exposing the stamen tube. Once the corolla lobes have separated sufficiently, the stamen tube elongates rapidly (T2). By the time the stamen tube is fully extended, and beginning to present pollen, the corolla lobes are usually fully spread, and slightly curved outwards so that the corolla forms a deep cup (T3). When all the pollen has been dispersed, the top of the style arms are visible level with, or just above the top of the stamen tube. As the floret enters the female phase the corolla begins to collapse, so that by the end of the female phase the outline of the corolla from above is that of a narrow ellipse. Once the style is clear of the stamen tube, the stamen tube rapidly withdraws into the corolla tube, so that the top is nearly level with the top of the corolla. As the stamen tube withdraws it splits down one side. As the stamen tube begins to withdraw, the style arms begin to separate and curve outwards (T4), so that by mid to late female phase the tips of the style arms usually point downwards (T5). The corolla of the tubular floret appears to withdraw before the style. By the time the style begins to brown, the corolla may be hidden by the pappus, or only visible as a series of corolla lobes. The style browns from the end of the arms first (T6), and is often completely brown before the corolla begins to brown (T7).

Raoulia tenuicaulis (Figure 3.48)

Filiform: The corolla of the filiform floret splits while the capitulum still appears to be in the bud stage (F1). The style elongates so that the tips of the style arms are the first floral structure to become visible (F2). The style continues to elongate throughout anthesis (F3, F4,), so that when fully extended the junction of the style arms is visible above the top of the involucral bracts (F5). As the style elongates the style arms gradually curl, and may eventually almost touch the style at the junction of the style arms (F5). The style and corolla appear to brown at approximately the same time, with the style usually browning in one style arm first (F6). The style usually withdraws slightly as it browns (F7).

Tubular: The corolla of the tubular floret begins to swell while still well below the level of the involucral bracts (T1, T2). When the swollen portion of the floret is above the top of

the involucral bracts the corolla begins to split from the top (T3). Once the corolla lobes have started to split, the lobes spread rapidly (T4), so that before the stamen tube is fully extended the corolla lobes are fully spread, and have curled under (T5). Pollen presentation (T6) appears to begin just before the stamen tube is fully extended. When all the pollen has been dispersed the stamen tube begins to split down one side. As the stamen tube splits, it withdraws a short distance back into the corolla. At this stage the tips of the style arms are usually visible just below the level of the stamen tube. During the "female phase" the style arms may spread slightly, forming a narrow V, or they may remain tightly closed together. This appeared to be population specific, with the styles arms of the plants at Dry Stream separating (T8), while those at Cass, and Broad Stream were observed to separate only a small distance, or not at all. The corolla of the tubular floret often begins to brown while the floret is still in the female phase, and has often collapsed by mid female phase. The style usually browns after the corolla, withdrawing into the corolla slightly as it browns (T9).

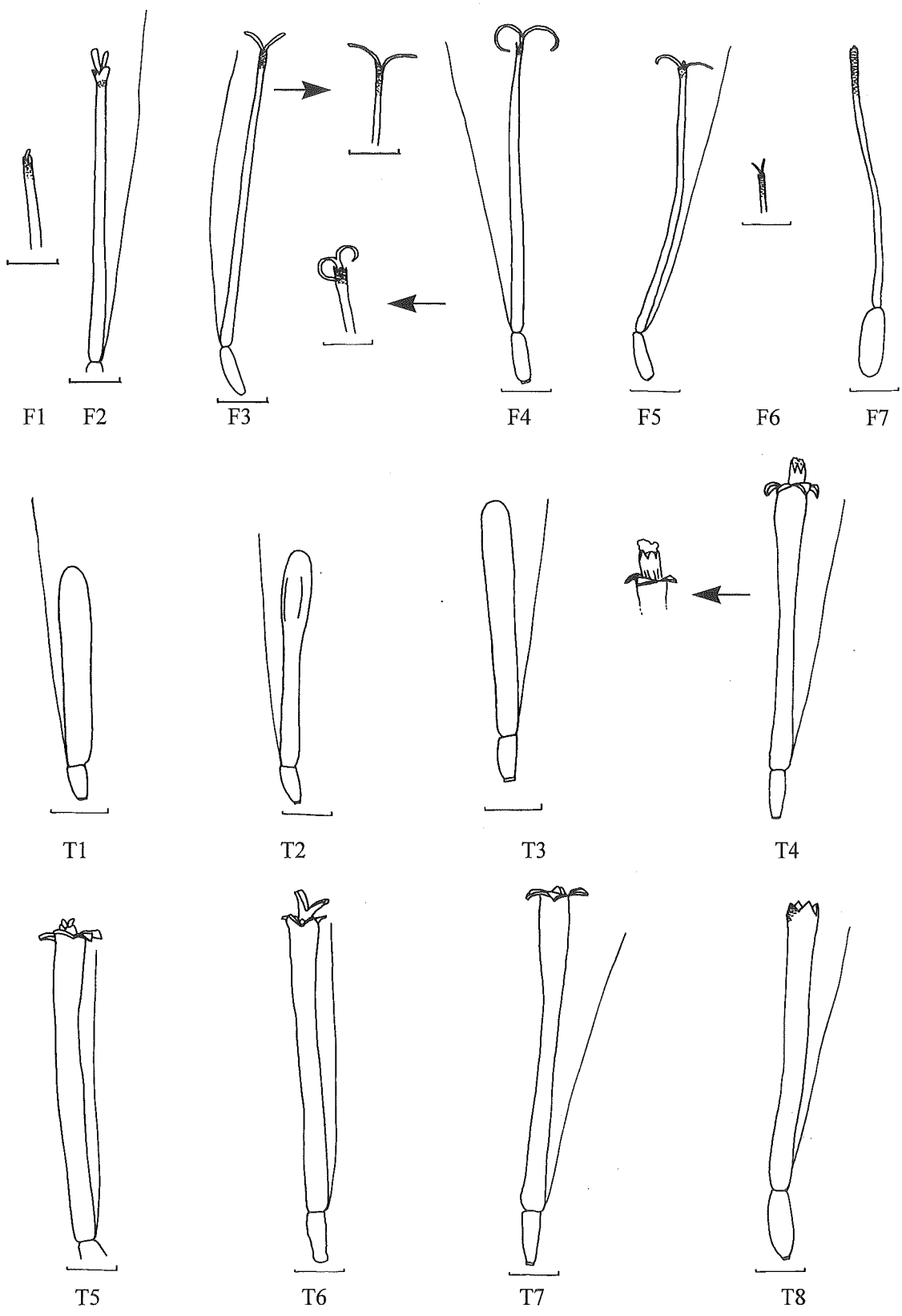


Figure 3.33: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Gnaphalium audax*. Scale equals 0.5 mm.

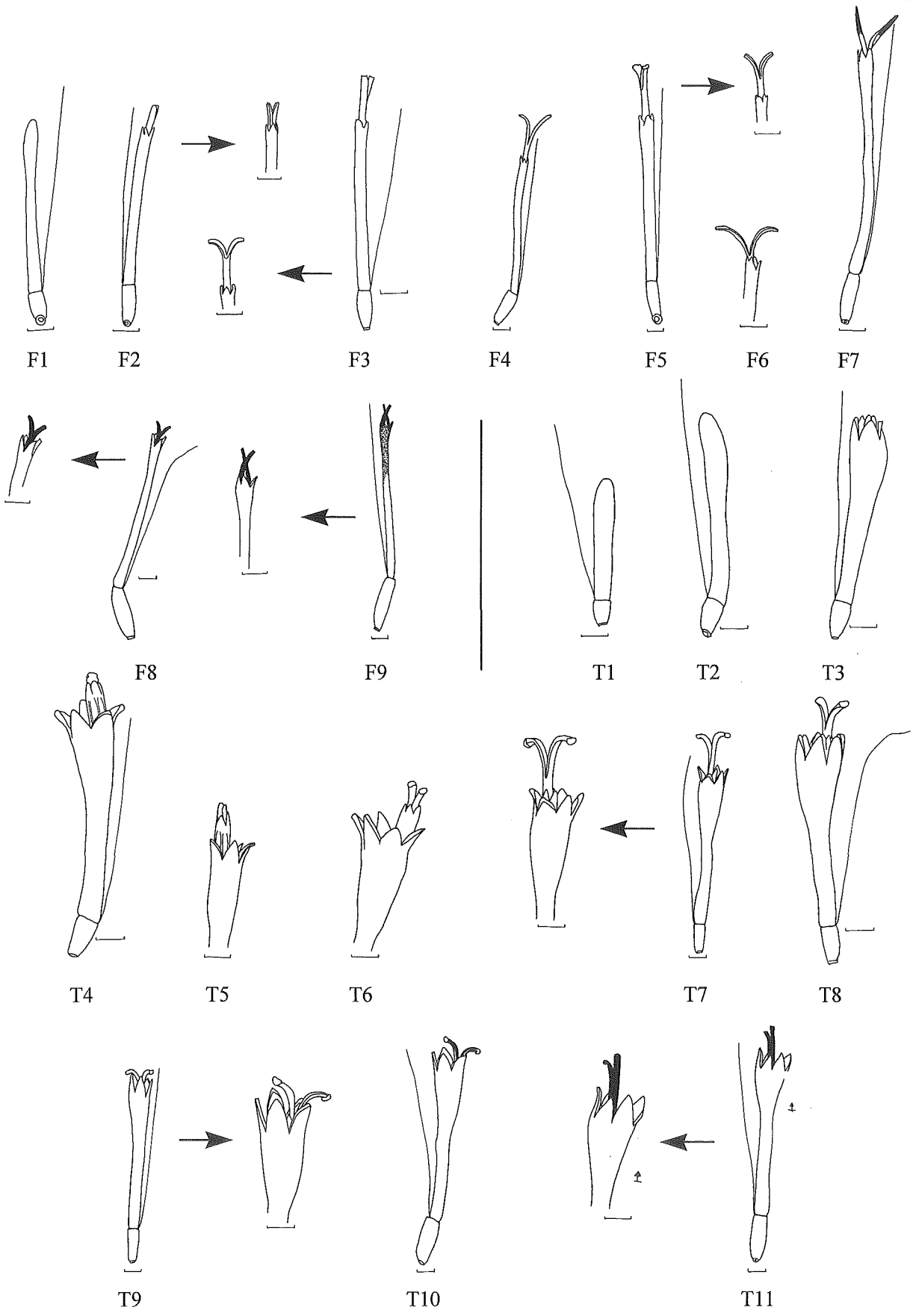


Figure 3.34: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Helichrysum bellidioides.* Scale equals 0.5 mm.

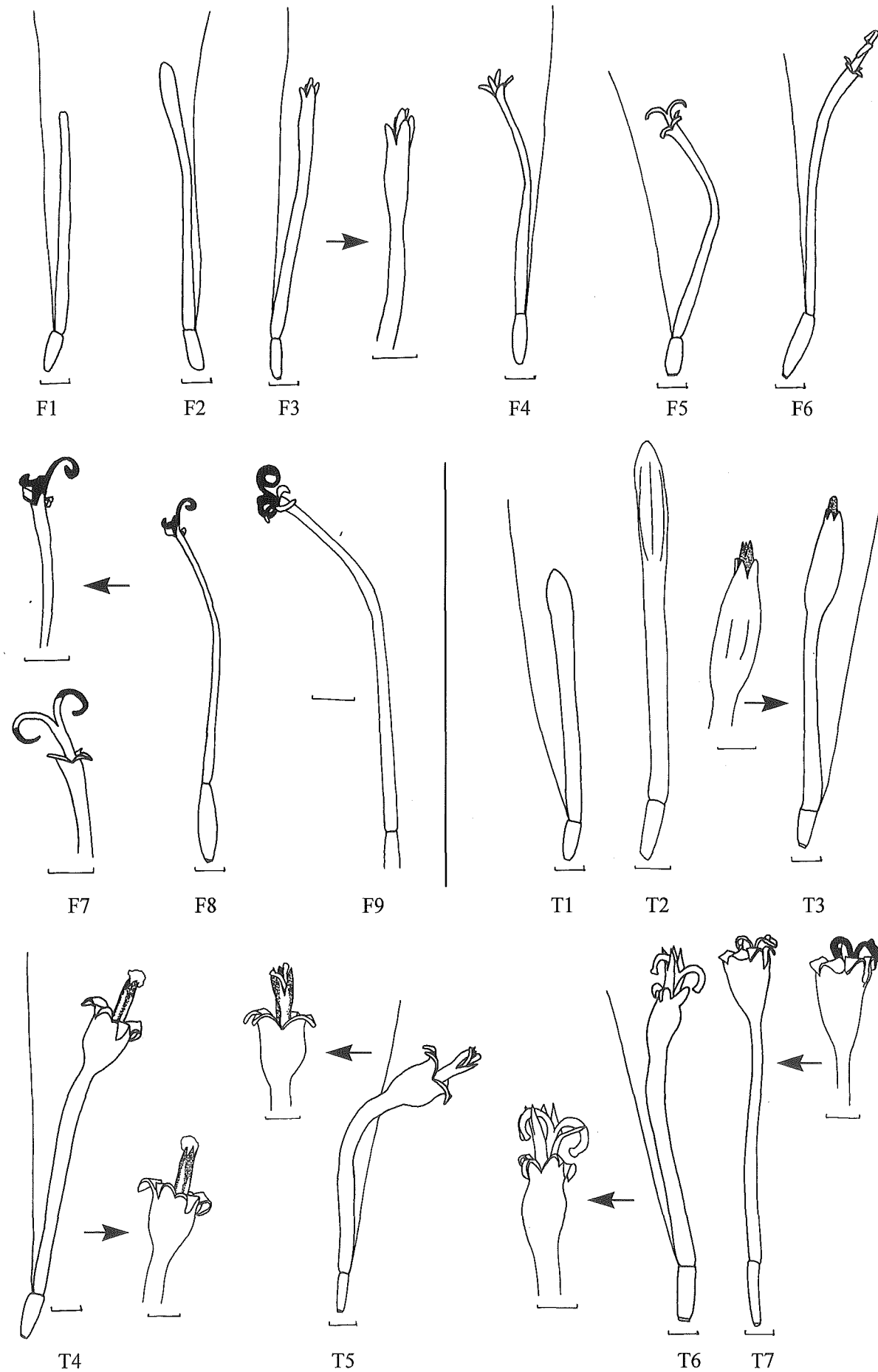


Figure 3.35: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Helichrysum filicaule.* Scale equals 0.5 mm.

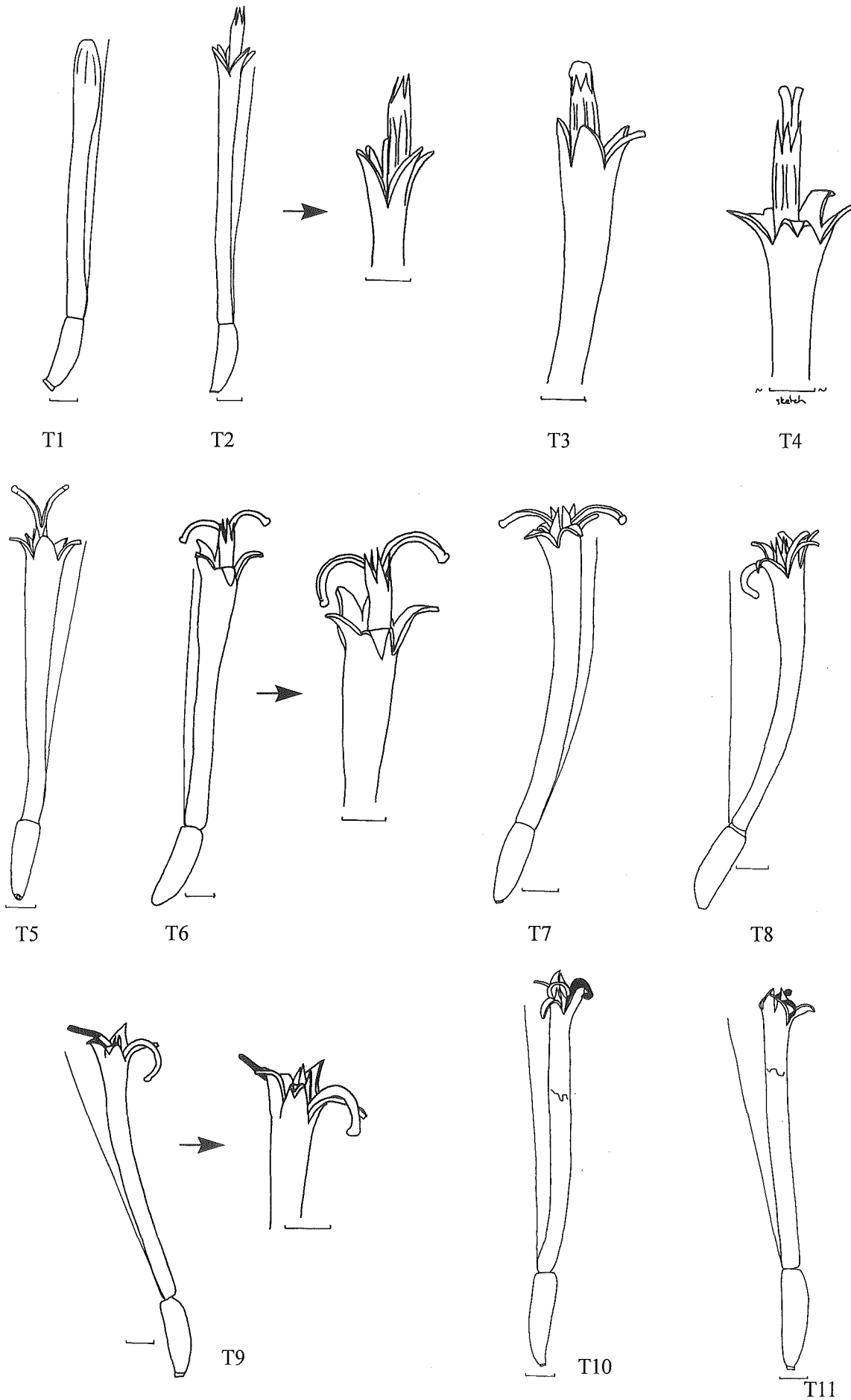


Figure 3.36: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Helichrysum depressum.* Scale equals 0.5 mm.

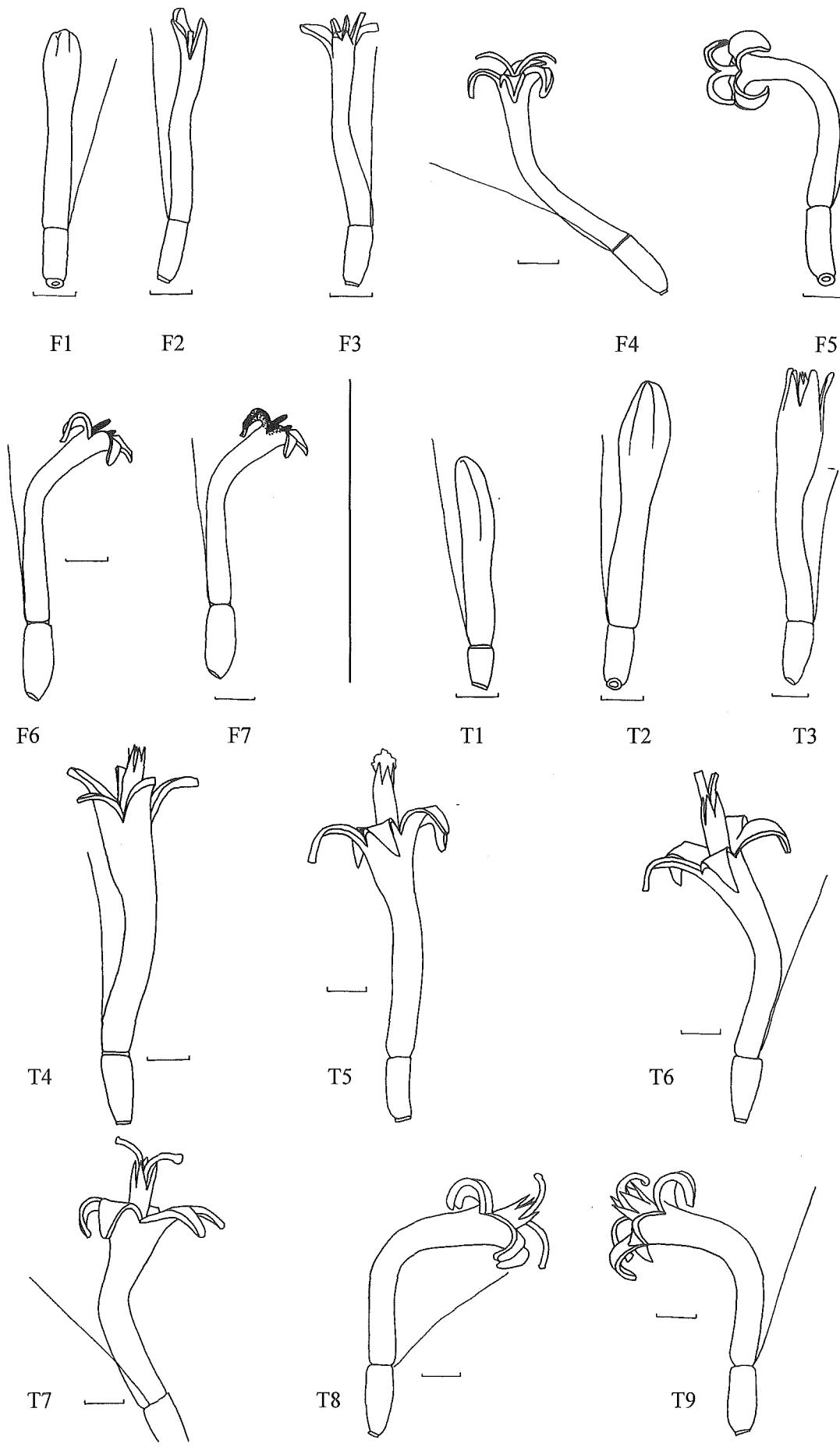


Figure 3.37: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Helichrysum intermedium*. Scale equals 0.5 mm.

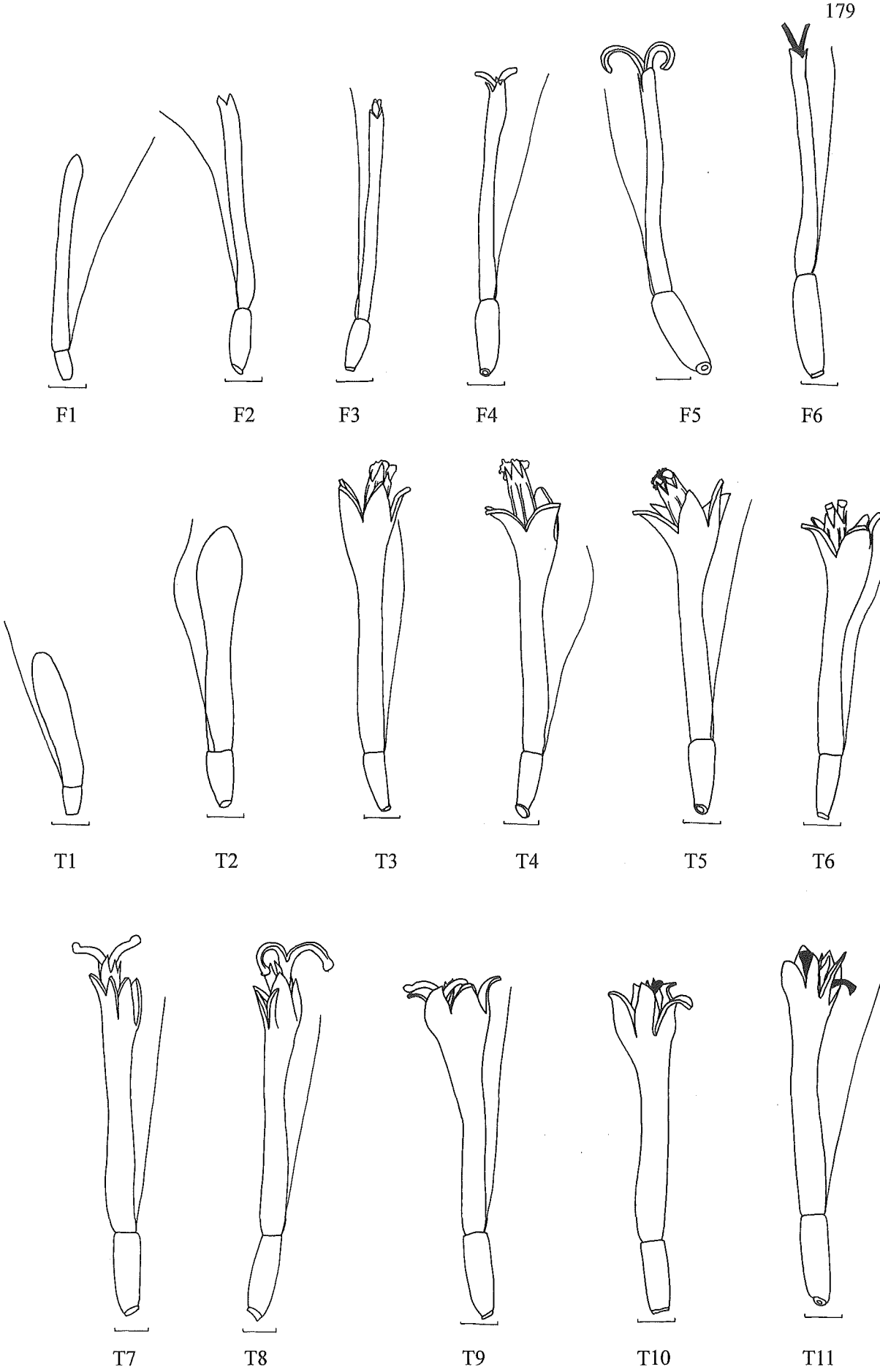


Figure 3.38: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Leucogenes grandiceps.* Scale equals 0.5 mm.

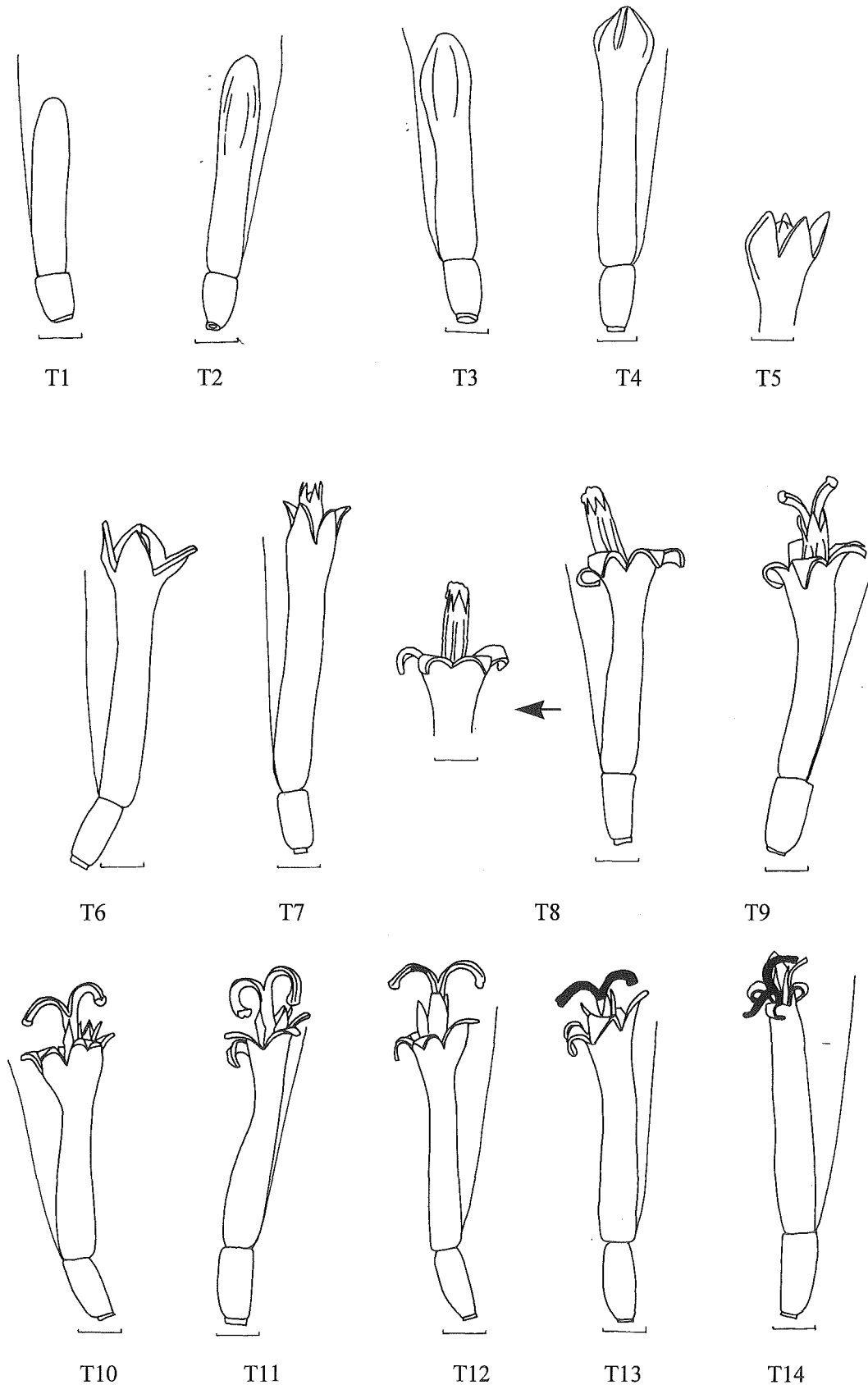


Figure 3.39: The phenological stages of the tubular florets during anthesis in *Ozothamnus leptophyllus.* Scale equals 0.5 mm.

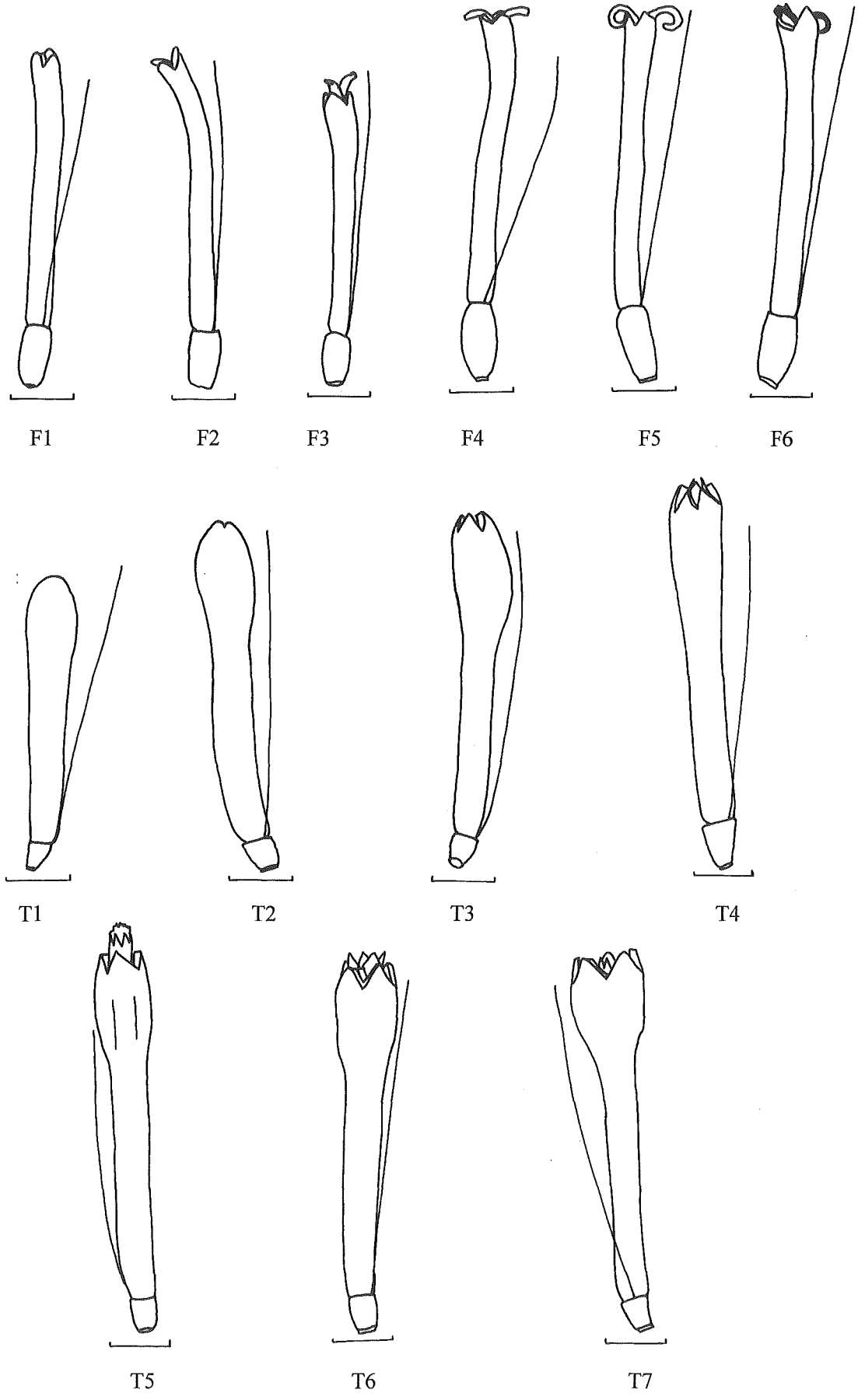


Figure 3.40: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia australis*. Scale equals 0.5 mm.

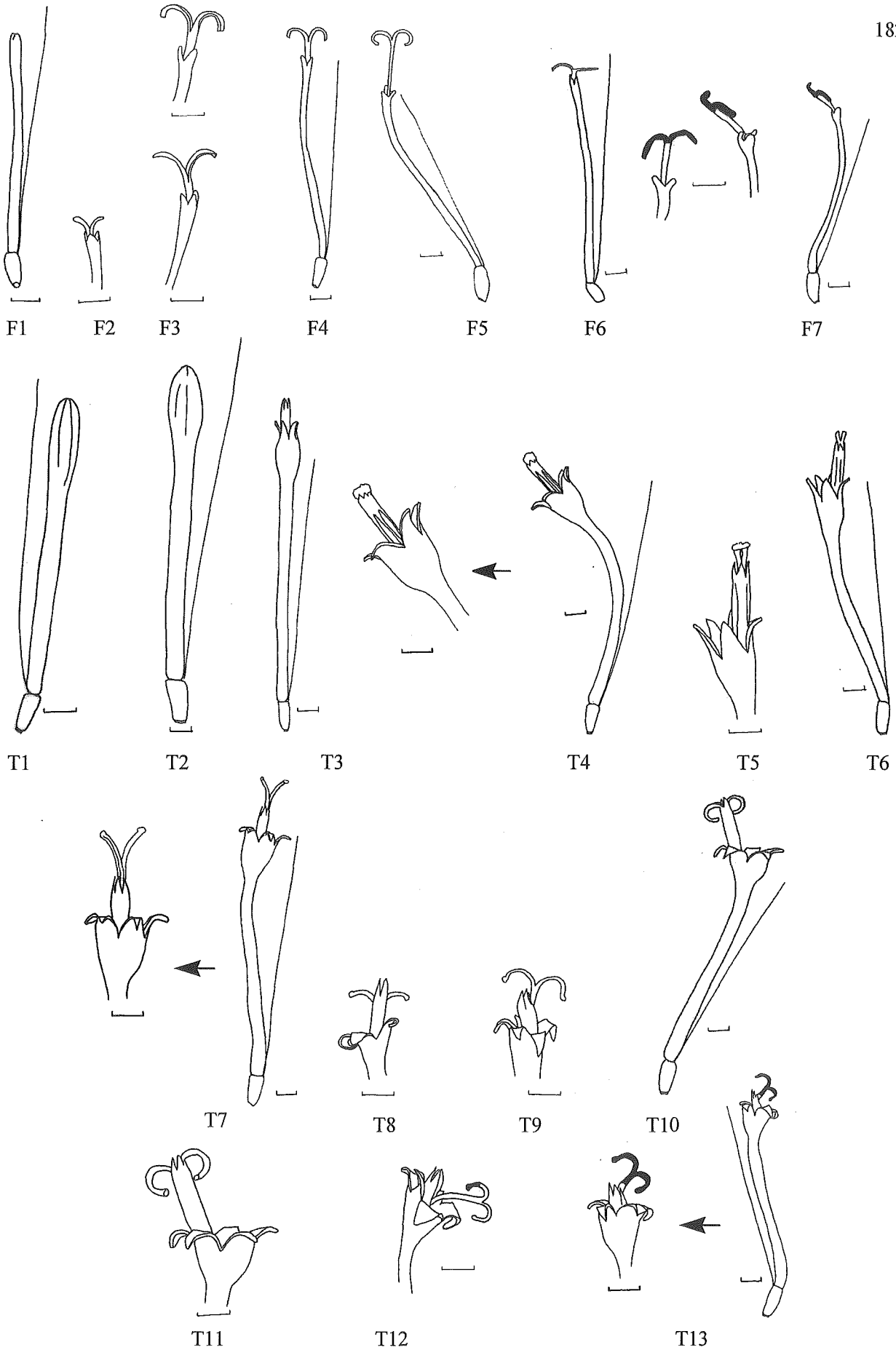


Figure 3.41: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia glabra.* Scale equals 0.5 mm.

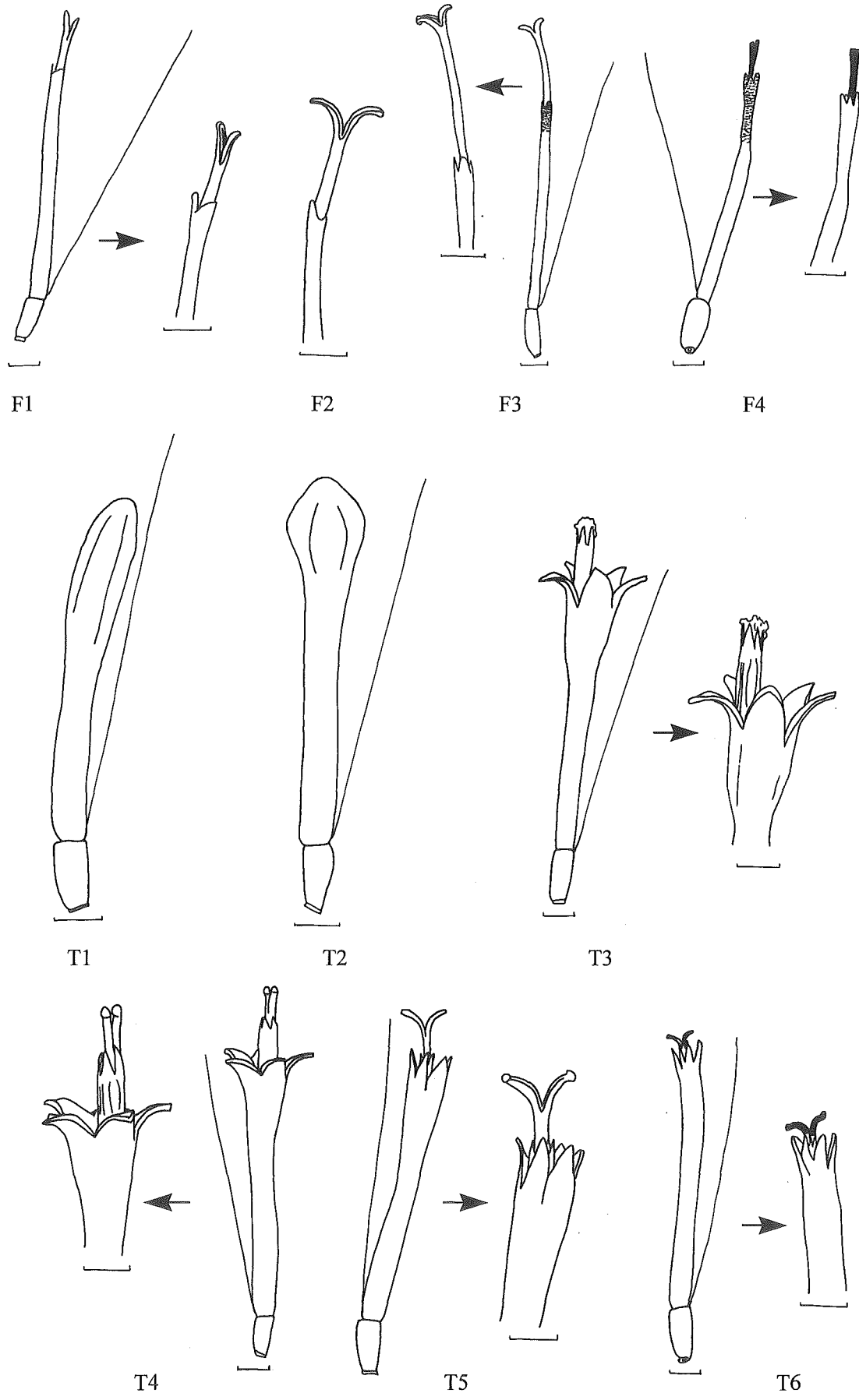


Figure 3.42: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia grandiflora*. Scale equals 0.5 mm.

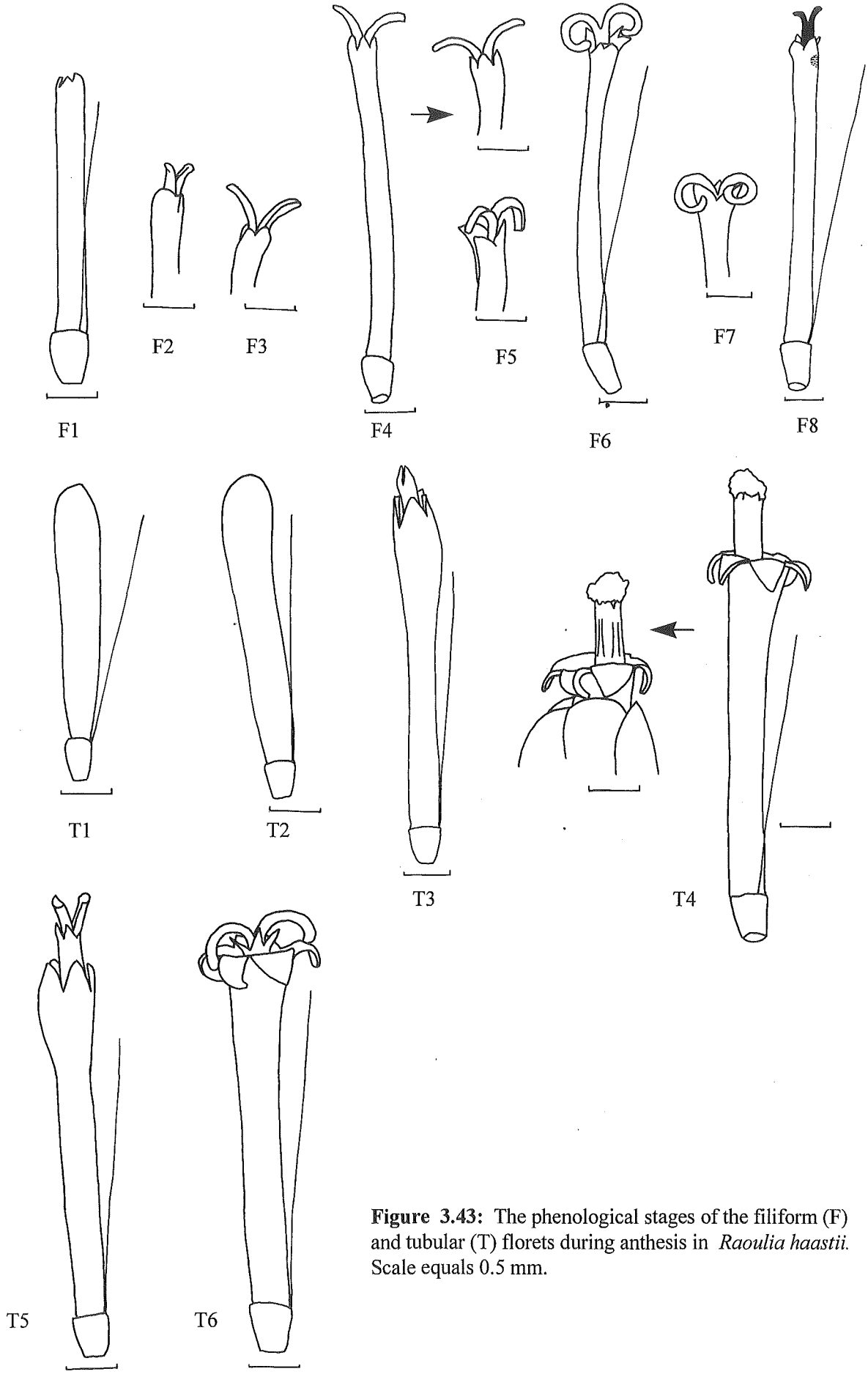


Figure 3.43: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia haastii*. Scale equals 0.5 mm.

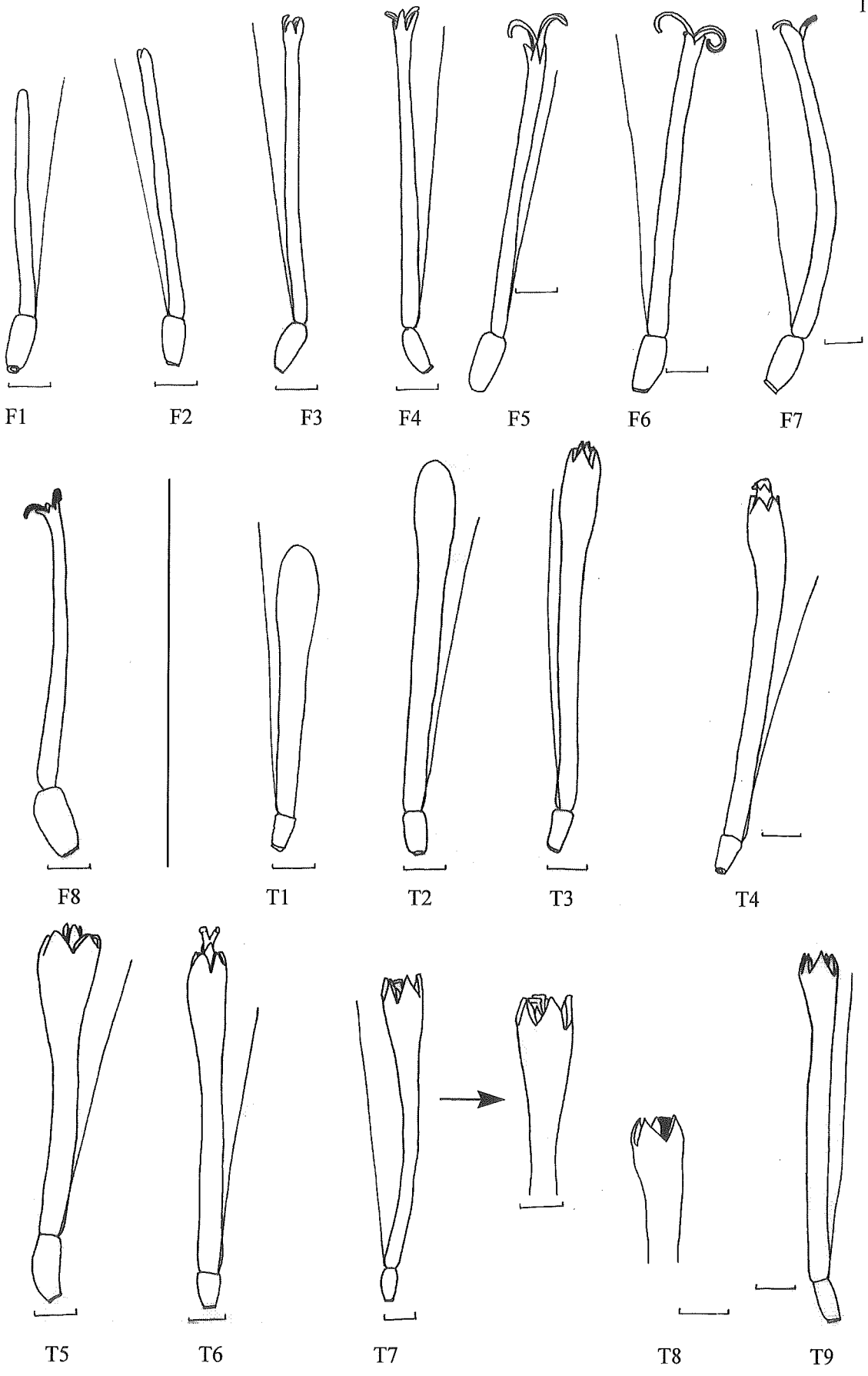


Figure 3.44: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia hookeri.* Scale equals 0.5 mm.

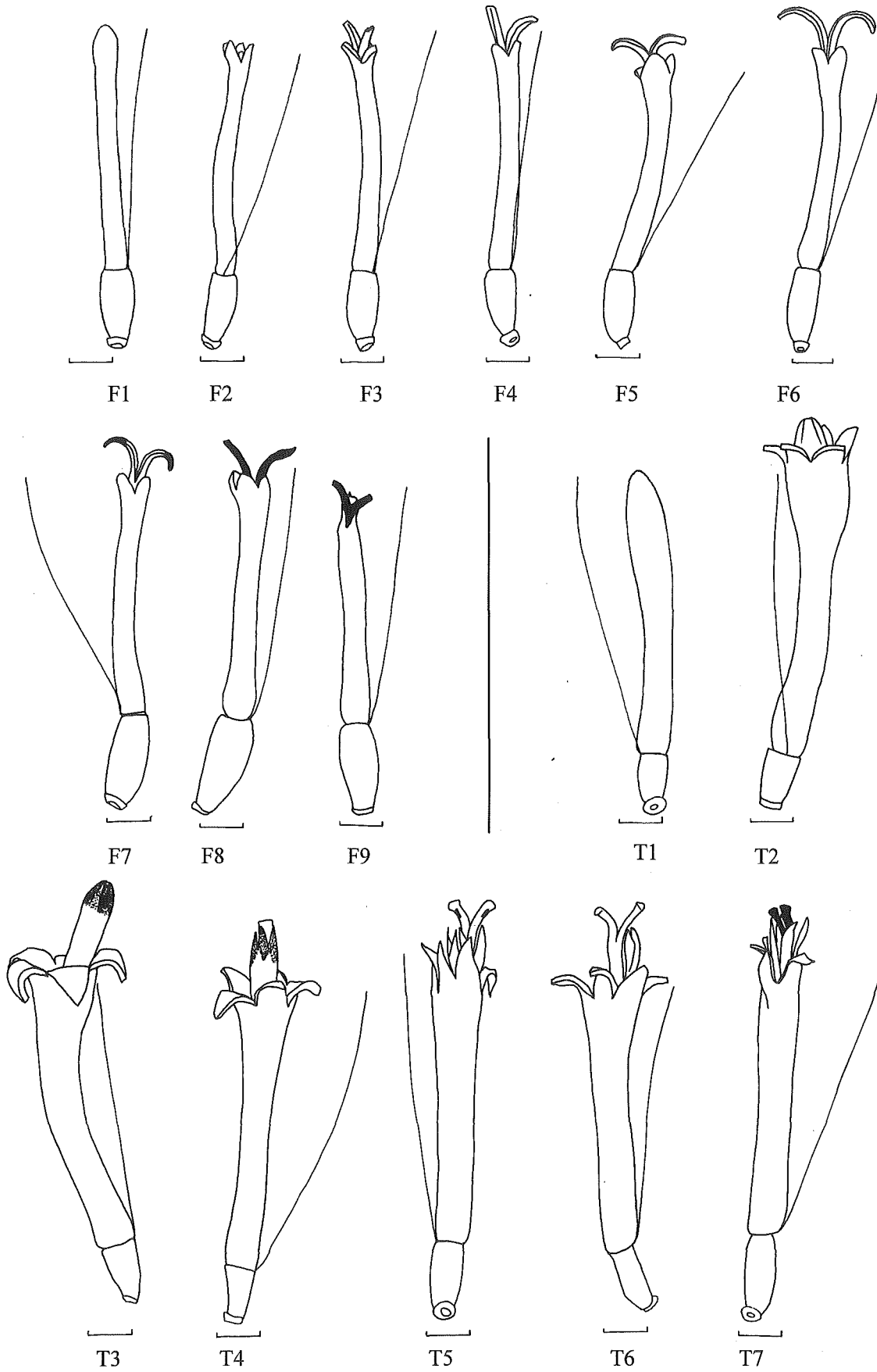


Figure 3.45: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia mammillaris*. Scale equals 0.5 mm.

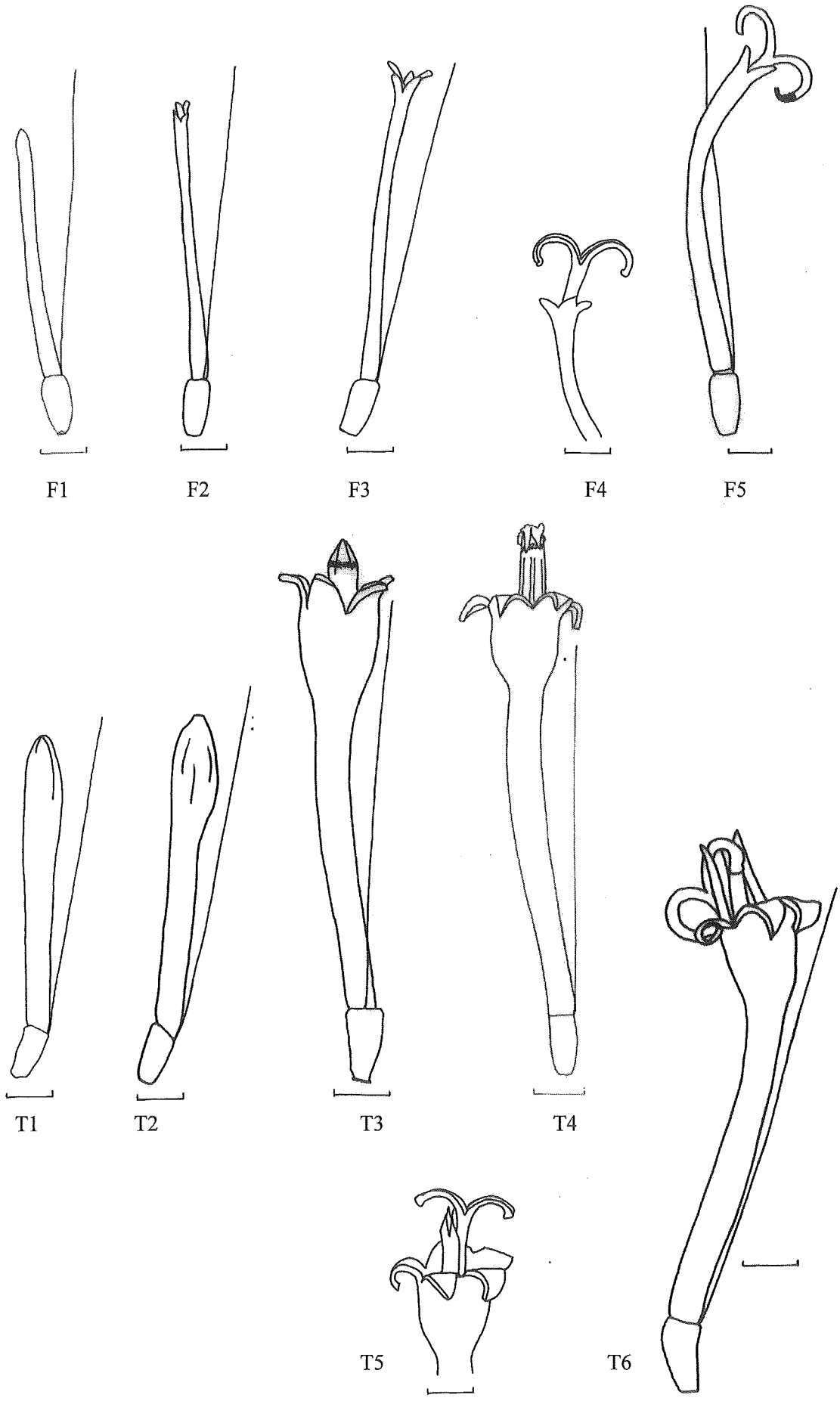


Figure 3.46: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia monroi*. Scale equals 0.5 mm.

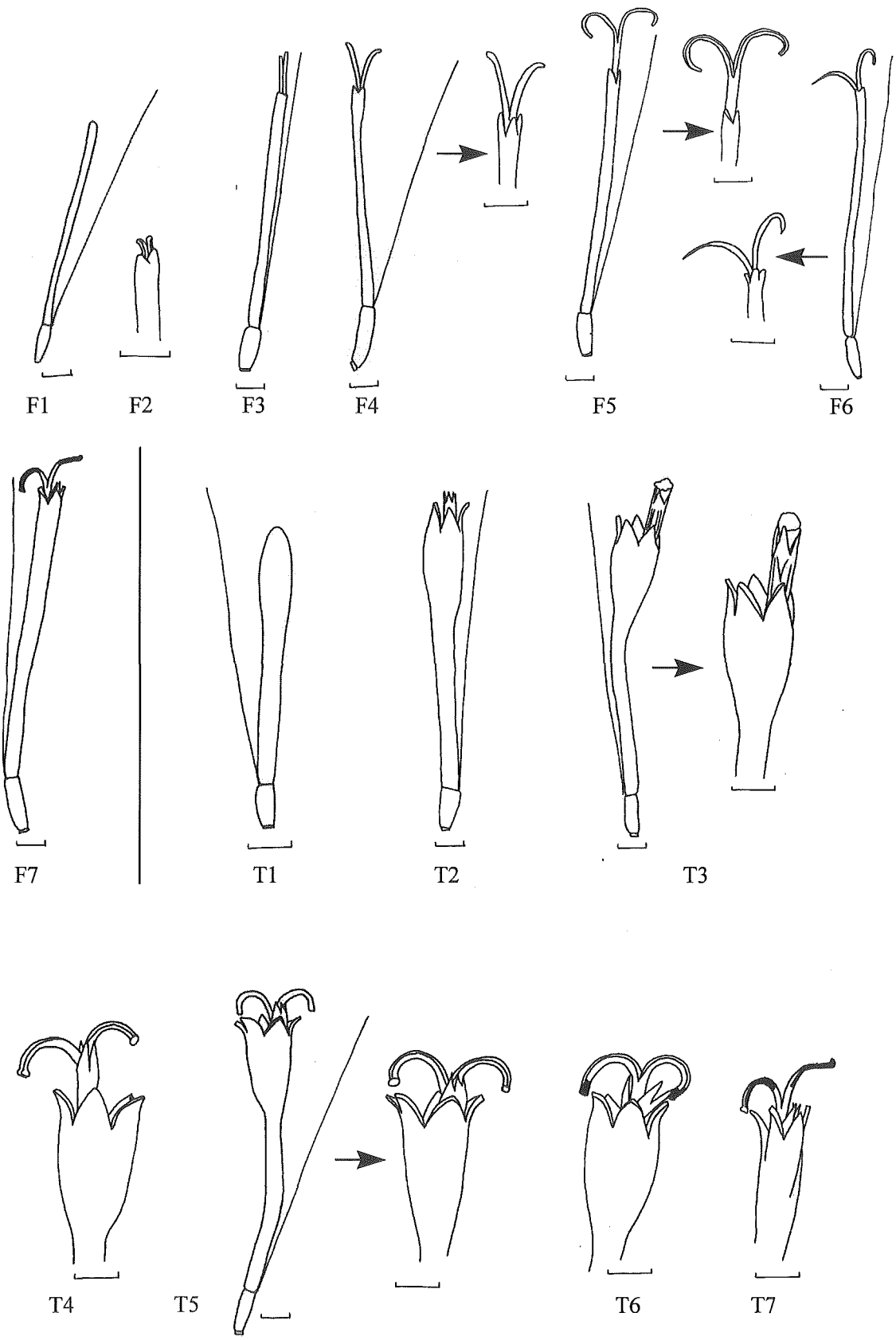


Figure 3.47: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia subsericea* . Scale equals 0.5 mm.

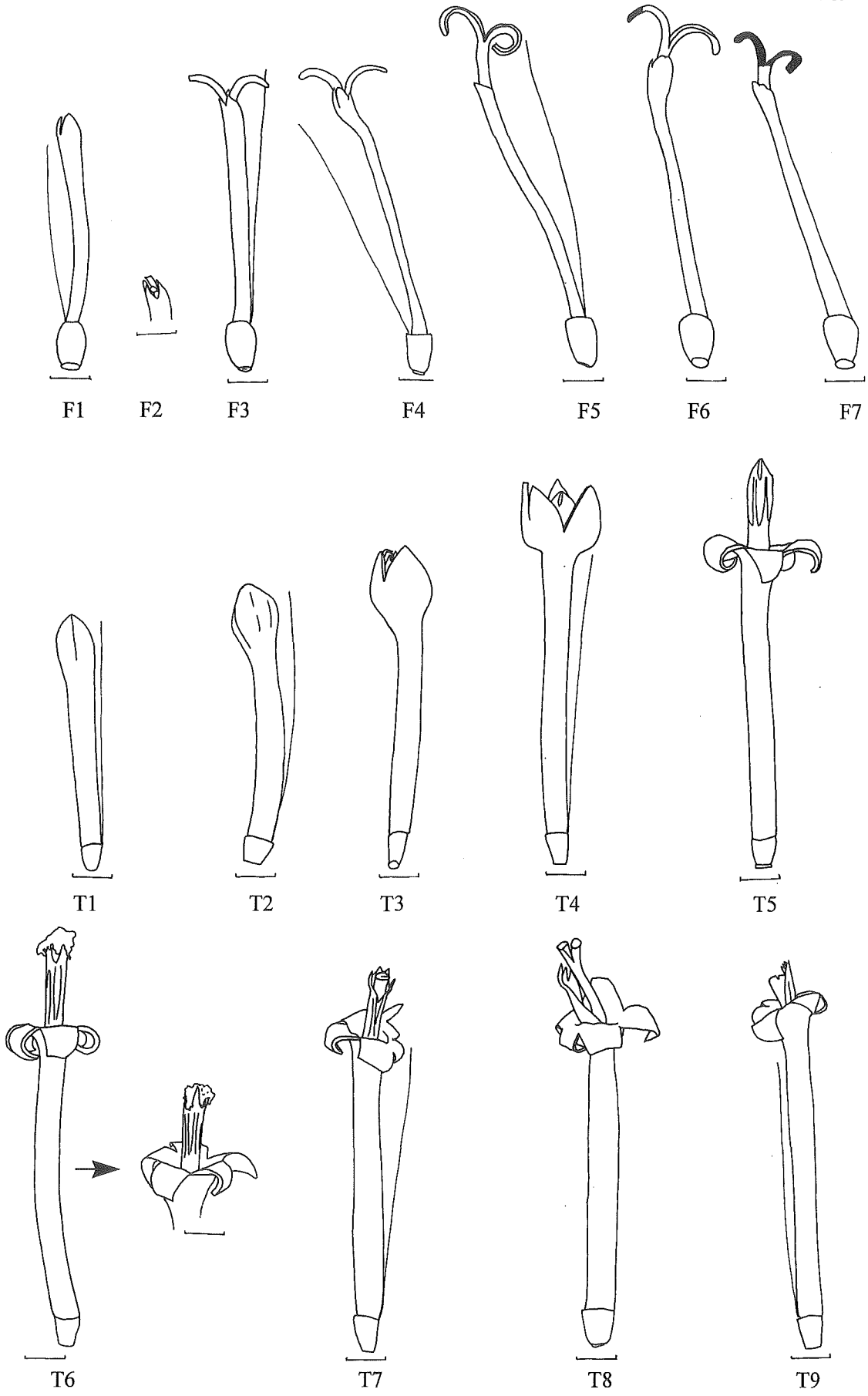


Figure 3.48: The phenological stages of the filiform (F) and tubular (T) florets during anthesis in *Raoulia tenuicaulis*. Scale equals 0.5 mm.

3.4.6 Breeding system

The average number of filiform and tubular florets per capitulum are summarised in Table 3.2. The ratio of filiform to tubular florets (i.e. the floret ratio) indicates that most species had a preponderance of tubular florets. A greater number of filiform florets was only observed in five species; *Gnaphalium audax, G. traversii, Helichrysum bellidioides, Raoulia haastii*, and *R. tenuicaulis*. The floret ratio in *G. audax* and *G. traversii* was very filiform biased, with over 13 filiform florets observed for every tubular floret. By contrast the floret ratio of *R. hookeri, R. subsericea, R. haastii,* and *H. bellidioides* was almost equal (Table 3.2). The floret ratio for *Ozothamnus leptophyllus* and *H. depressum* is equal to zero, since no filiform florets were observed in either species (Table 3.2). The three species with the greatest number of florets (*G. audax, G. traversii*, and *H. bellidioides*) all had majority of filiform florets, while most species with smaller capitula were observed to have a more even, or tubular biased, floret ratio (the exceptions were *R. haastii* and *R. tenuicaulis*). The species with the smallest capitula was *R. haastii*, with an average of 4.3 florets per capitulum (Table 3.2)

Species	F florets	T florets	Floret ratio	Total florets	N
Gnaphalium audax	51.0 ± 4.1	3.7 ± 0.7	13.14 ± 2.21	54.9 ± 4.4	32
Gnaphalium traversii	121.0 ± 27.4	7.8 ± 2.2	15.93 ± 3.04	128.8 ± 28.4	28
Helichrysum bellidioides	97.4 ± 18.0	82.9 ± 14.1	1.18 ± 0.14	180.3 ± 30.5	34
Helichrysum depressum	0 ± 0	11.3 ± 1.9	0 ± 0	11.3 ± 1.9	32
Helichrysum filicaule	15.4 ± 2.3	20.9 ± 5.1	0.75 ± 0.10	36.3 ± 7.0	24
Helichrysum intermedium	10.8 ± 2.1	23.9 ± 5.0	0.46 ± 0.07	34.7 ± 6.7	30
Leucogenes grandiceps	10.4 ± 1.2	19.2 ± 4.6	0.56 ± 0.10	29.6 ± 5.5	31
Ozothamnus leptophyllus	0 ± 0	7.3 ± 2.0	0 ± 0	7.3 ± 2.0	31
Raoulia australis	3.4 ± 0.7	5.1 ± 0.6	0.67 ± 0.14	8.4 ± 1.0	31
Raoulia glabra	13.3 ± 2.5	30.9 ± 4.9	0.43 ± 0.05	44.2 ± 7.0	34
Raoulia grandiflora	11.6 ± 2.1	20.2 ± 4.2	0.58 ± 0.08	31.9 ± 6.1	21
Raoulia haastii	2.3 ± 0.5	2.0 ± 0.4	1.20 ± 0.52	4.3 ± 0.5	40
Raoulia hookeri	9.0 ± 2.3	11.3 ± 4.0	0.83 ± 0.17	20.4 ± 6.0	30
Raoulia mammillaris	4.4 ± 0.8	6.6 ± 1.2	0.69 ± 0.18	11.1 ± 1.5	33
Raoulia monroi	4.7 ± 1.2	8.0 ± 1.1	0.60 ± 0.17	12.6 ± 1.6	23
Raoulia subsericea	15.8 ± 3.8	17.8 ± 2.3	0.88 ± 0.18	33.6 ± 5.4	25
Raoulia tenuicaulis	5.5 ± 0.7	3.8 ± 0.6	1.48 ± 0.38	9.4 ± 1.0	31

Table 3.2: The number of filiform (F), tubular (T) and total florets in the capitula and the ratio of filiform to tubular florets. (mean ± standard deviation). (N equals sample size.)

The proportion of seed set by filiform florets ranged from 3% in *Raoulia monroi* to nearly 90% in *Gnaphalium audax, G. traversii* and *R. mammillaris* (Table 3.3). By comparison the proportion of tubular florets setting seed ranged from 0% in *R. hookeri* and

R. tenuicaulis, to 73% in *G. traversii*. The ratio of proportion of seed set by filiform to tubular florets ranged from 1.19, in *G. traversii*, to over 68 in *R. australis* (Table 3.3). This indicates that the proportion of filiform florets setting seed is greater than the proportion of tubular setting seed in all species. This ratio is presented as ∞ for *R. hookeri* and *R. tenuicaulis*, since no tubular were observed to set seed. Conversely, the ratio for *Helichrysum depressum* and *Ozothamnus leptophyllus* is 0, since no filiform florets were observed (Table 3.3). The proportion of all florets in a capitulum setting seed indicates that most species set seed in 20% to 40% of florets (Table 3.3). The lowest proportion of florets observed setting seed was 2%, in *R. monroi*, while the highest value was observed in the two *Gnaphalium* species, each setting seed in 87% of florets.

Species	proportion F set seed	proportion of T seed set	ratio F:T	Proportion of all florets setting seed	N
Gnaphalium audax	0.895 ± 0.098	0.553 ± 0.305	1.619	0.870 ± 0.091	30
Gnaphalium traversii	0.879 ± 0.247	0.734 ± 0.328	1.196	0.870 ± 0.247	28
Helichrysum bellidioides	0.689 ± 0.248	0.163 ± 0.195	4.233	0.451 ± 0.183	31
Helichrysum depressum	0 ± 0	0.369 ± 0.334	0	0.369 ± 0.334	32
Helichrysum filicaule	0.267 ± 0.305	0.161 ± 0.216	1.664	0.206 ± 0.246	24
Helichrysum intermedium	0.563 ± 0.274	0.429 ± 0.201	1.313	0.472 ± 0.177	30
Leucogenes grandiceps	0.493 ± 0.241	0.293 ± 0.214	1.684	0.364 ± 0.199	31
Ozothamnus leptophyllus	0 ± 0	0.087 ± 0.184	0	0.087 ± 0.184	31
Raoulia australis	0.441 ± 0.307	0.006 ± 0.036	68.333	0.183 ± 0.127	31
Raoulia glabra	0.287 ± 0.297	0.113 ± 0.124	2.546	0.166 ± 0.150	12
Raoulia grandiflora	0.490 ± 0.348	0.303 ± 0.172	1.617	0.367 ± 0.191	21
Raoulia haastii	0.504 ± 0.255	0.175 ± 0.269	2.881	0.360 ± 0.184	40
Raoulia hookeri	0.595 ± 0.403	0 ± 0	∞	0.268 ± 0.181	30
Raoulia mammillaris	0.873 ± 0.135	0.020 ± 0.048	44.145	0.361 ± 0.078	33
Raoulia monroi	0.026 ± 0.092	0.021 ± 0.060	1.234	0.023 ± 0.067	23
Raoulia subsericea	0.196 ± 0.150	0.042 ± 0.065	4.68	0.108 ± 0.082	23
Raoulia tenuicaulis	0.480 ± 0.281	0 ± 0	∞	0.288 ± 0.185	31

Table 3.3: The proportion of filiform (F) and tubular (T) florets setting seed, the ratio of F:T florets setting seed, and the proportion of total florets setting seed. (mean ± standard deviation. N equals sample size.)

The number of pollen grains per tubular floret ranged from just under 500 in *Gnaphalium audax*, to over 2 500 in *Leucogenes grandiceps* (Table 3.4). All species except *G. audax, G. traversii* and *Raoulia haastii* were observed to contain, on average, over 1 000 pollen grains per tubular floret. Most species were found to be reasonably constant in the number of pollen grains per tubular floret, as indicated by the low standard deviations, and contain between 1 200 and 1 900 pollen grains (Table 3.4). The pollen of *Raoulia mammillaris* appeared to differ from the other species examined, with a large number of small pollen

grains (i.e. grains approximately half the size of the normal grains). The number of small grains in *R. mammillaris* was estimated by counting the number of small grains in the first 100 pollen grains encountered in each of the six total pollen counts. This estimate indicated that from 29% to 60% (mean = 43%) of the pollen grains in *R. mammillaris* were small. In the other species the number of small pollen grains was not counted as only a few percent of the of pollen grains were observed to be small. In these species no more than 10-15 small pollen grains were observed per floret.

The pollen-ovule (p/o) ratios of *Gnaphalium traversii* and *G. audax* are distinct from all other species examined (Figure 3.49), with p/o ratios of approximately 35 (Table 3.4). All other species had values between 434, in *R. mammillaris*, and 1 739 pollen grains per ovule, in *Leucogenes grandiceps* (Table 3.4). The p/o for *Raoulia tenuicaulis* and *R. hookeri* are probably an underestimate of the true p/o ratio, as the tubular florets set no seed in these species. Thus, if the tubular floret ovules are removed from the calculation the p/o ratio for these species approximately doubles to 824 and 2 392, for *R. tenuicaulis* and *R. hookeri* respectively. The value of *L. grandiceps* is also probably an underestimate, as the central capitula in the cluster contains a higher proportion of tubular florets than the outer capitula (pers. obs.).

Species	Pollen grains per floret	P/O ratio
Gnaphalium audax	479.2 ± 60.4 (6)	34.6 ± 4.3
Gnaphalium traversii	576.5 ± 126.5 (6)	35.0 ± 8.3
Helichrysum bellidioides	1792.8 ± 254.1 (6)	824.6 ± 116.7
Helichrysum depressum	1659.0 ± 128.1 (6)	1659.0 ± 208.8
Helichrysum filicaule	1260.8 ± 93.2 (6)	725.9 ± 124.1
Helichrysum intermedium	1363.7 ± 96.6 (6)	937.9 ± 139.0
Leucogenes grandiceps	2678.5 ± 29.6 (6)	1739.0 ± 258.7
Ozothamnus leptophyllus	1629.5 ± 246.9 (6)	1629.5 ± 351.5
Raoulia australis	1645.7 ± 96.6 (6)	989.9 ± 83.5
Raoulia glabra	1329.7 ± 149.1 (6)	928.9 ± 118.3
Raoulia grandiflora	2101.8 ± 48.3 (6)	1335.3 ± 188.4
Raoulia haastii	917.7 ± 79.1 (6)	434.7 ± 61.7
Raoulia hookeri	1906.8 ± 85.9 (6)	1061.1 ± 247.4
Raoulia mammillaris	1650.7 ± 274.1 (6)	990.4 ± 150.4
Raoulia monroi	1549.5 ± 110.4 (6)	977.8 ± 103.0
Raoulia subsericea	1416.0 ± 60.5 (4)	751.8 ± 74.6
Raoulia tenuicaulis	1184.5 ± 415.9 (6)	486.1 ± 90.1

Table 3.4: The average number of pollen grains per floret, and pollen-ovule ratio for the 17 species of Inuleae in the Cass-Craigieburn district. (mean ± standard deviation (sample size))

All species were observed to have sensitive stamens. When touched a stamen reacted in one of the two following ways: By (1) moving towards, then away from the stimulus, presenting more pollen during this process; (2) moving towards the stimulus and presenting more pollen. Each species was observed to usually only react in one of these ways. The second type of reaction was observed in *Helichrysum bellidioides, Raoulia haastii, R. hookeri,* and *R. glabra.* The first type of reaction was usually observed in the other ten species.

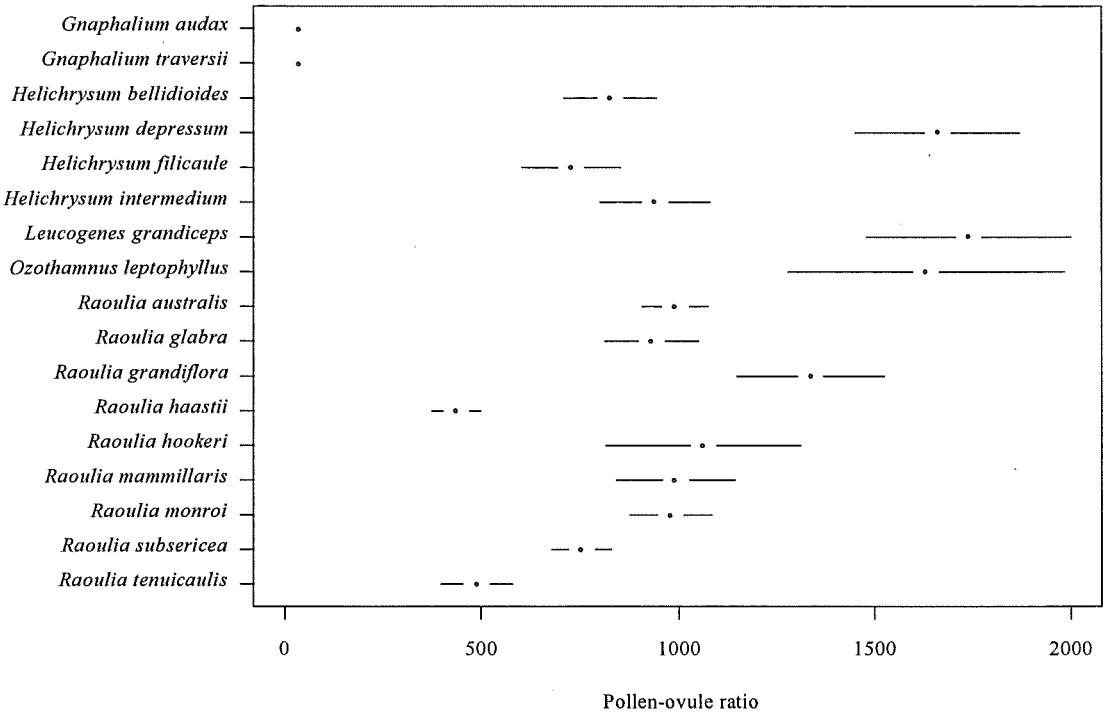


Figure 3.49: The average pollen-ovule ratios observed in samples from populations of 17 species of the New Zealand Inuleae in the Cass-Craigieburn district. (Horizontal bars indicate 1 standard deviation.)

The phenotypic and functional gender estimates for each species are depicted in Figure 3.50. Each point in the graphs represents the gender estimate for a single capitulum. The narrow spread of data points on the x-axis (i.e. the phenotypic gender) of each subplot indicates that in most species the capitula do not vary greatly in their phenotypic gender. By contrast, a wide spread of points is visible along the y-axis of each subplot (Figure 3.50), indicating that the capitula of most species do not all function equally as females. Most species contain some individual capitula that function only as males (i.e. they do not set seed) (e.g. Figure 3.50 m, n). Exceptions to these trends were *Gnaphalium audax, G. traversii, R. haastii,* and *R. mammillaris,* which have all the points clustered in the middle of their respective graphs (Figure 3.50 a, b, i, n), indicating that the capitula in

these species have a functional gender similar to their phenotypic gender. However, none of the plots contains a straight line relationship between phenotypic and functional gender, indicating that the slight difference in phenotypic gender does not correlate with the differences in functional gender.

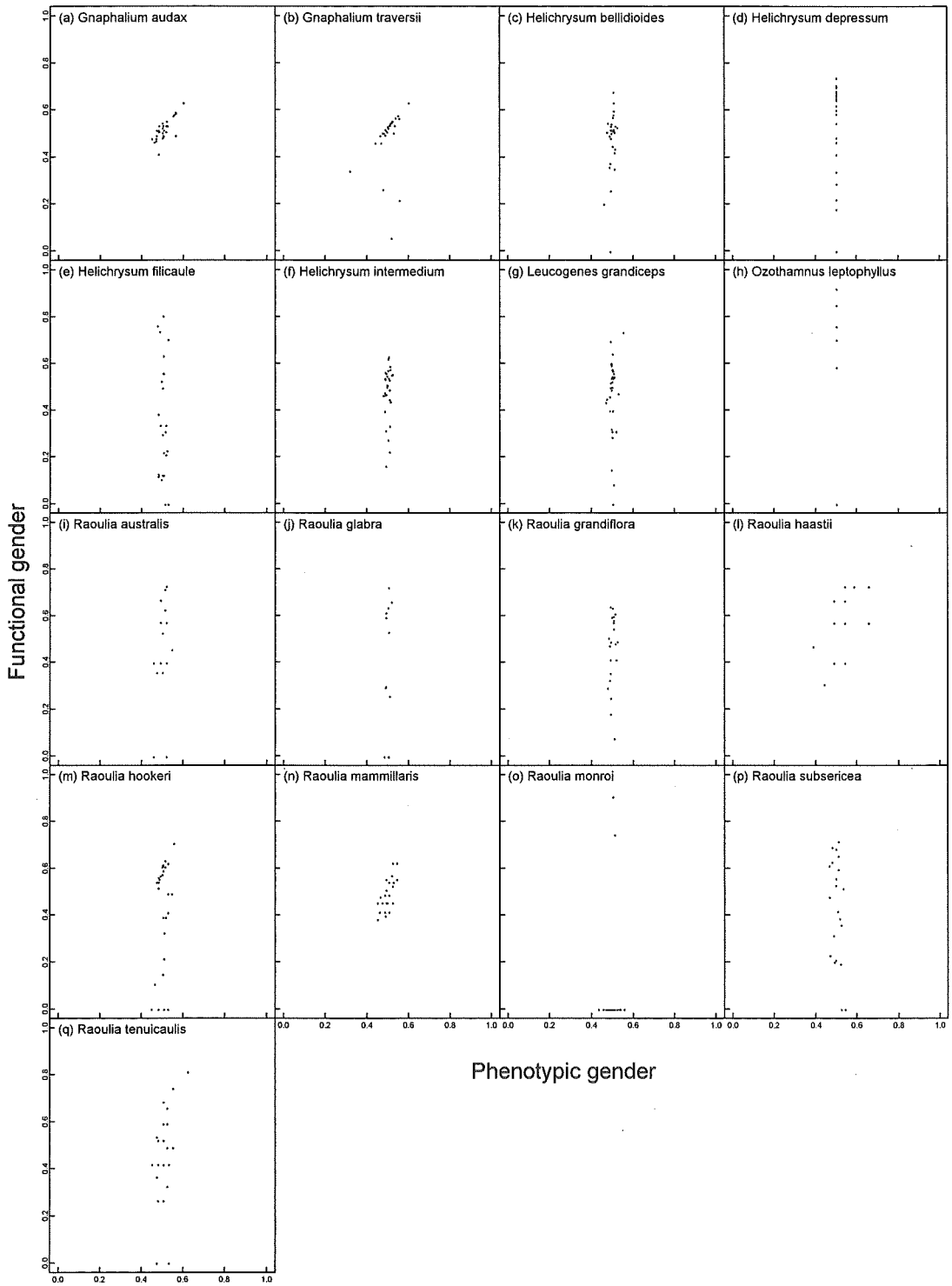


Figure 3.50: Phenotypic and functional female gender for each capitulum sampled in the 17 species of the Inuleae observed in the Cass-Craigieburn district.

The gender estimates calculated from the average pollen, seed and floret values for each species provide a comparative measure of the functional and phenotypic gender for each species (Figure 3.51). These values can not be used, however, as an absolute measure of gender, since the addition or removal of a species from the calculations alters the gender estimates. For example, the phenotypic and functional gender estimates for *Raoulia haastii* are 0.619 and 0.542 respectively (Figure 3.51). However, if *G. traversii* and *G. audax* are removed from the calculations theses values become 0.689 and 0.699, respectively. Despite altering the absolute value of the gender estimates, the addition or removal of species does not affect the position of the species relative to each other and is therefore a useful comparative measure.

The gender estimates for *Gnaphalium audax* and *G. traversii* are distinct from all other species, having a much greater female function compared to the other species, both in phenotypic and functional gender. *Raoulia haastii* and *R. tenuicaulis* also have slightly higher female phenotypic genders than most other species, but are only slightly more functionally female than *Helichrysum bellidioides* and *H. intermedium* (Figure 3.51). *Ozothamnus leptophyllus, Leucogenes grandiceps* and *H. depressum* all have low female phenotypic gender estimates, however the last two species have a functional gender that is approximately equal to most other species. Conversely, *R. monroi* has a phenotypic gender approximately equal to most other species, however, functionally, this species is the least female of all the species examined (Figure 3.51). Only two species were found to have a functional gender estimates which exceeded their phenotypic gender estimates. These were *G. audax* and *G. traversii*, both increasing from approximately 0.95 to 0.97 (Figure 3.51).

Scent was detected in all species except *Gnaphalium traversii* and *G. audax*. In all scented species, except *Raoulia mammillaris*, the scent was a pleasant sweet smell which was particularly strong in some species (e.g. *Helichrysum filicaule, H. depressum*). The scent of *R. mammillaris* was not as sweet as the other species, and had an unpleasant element to the smell.

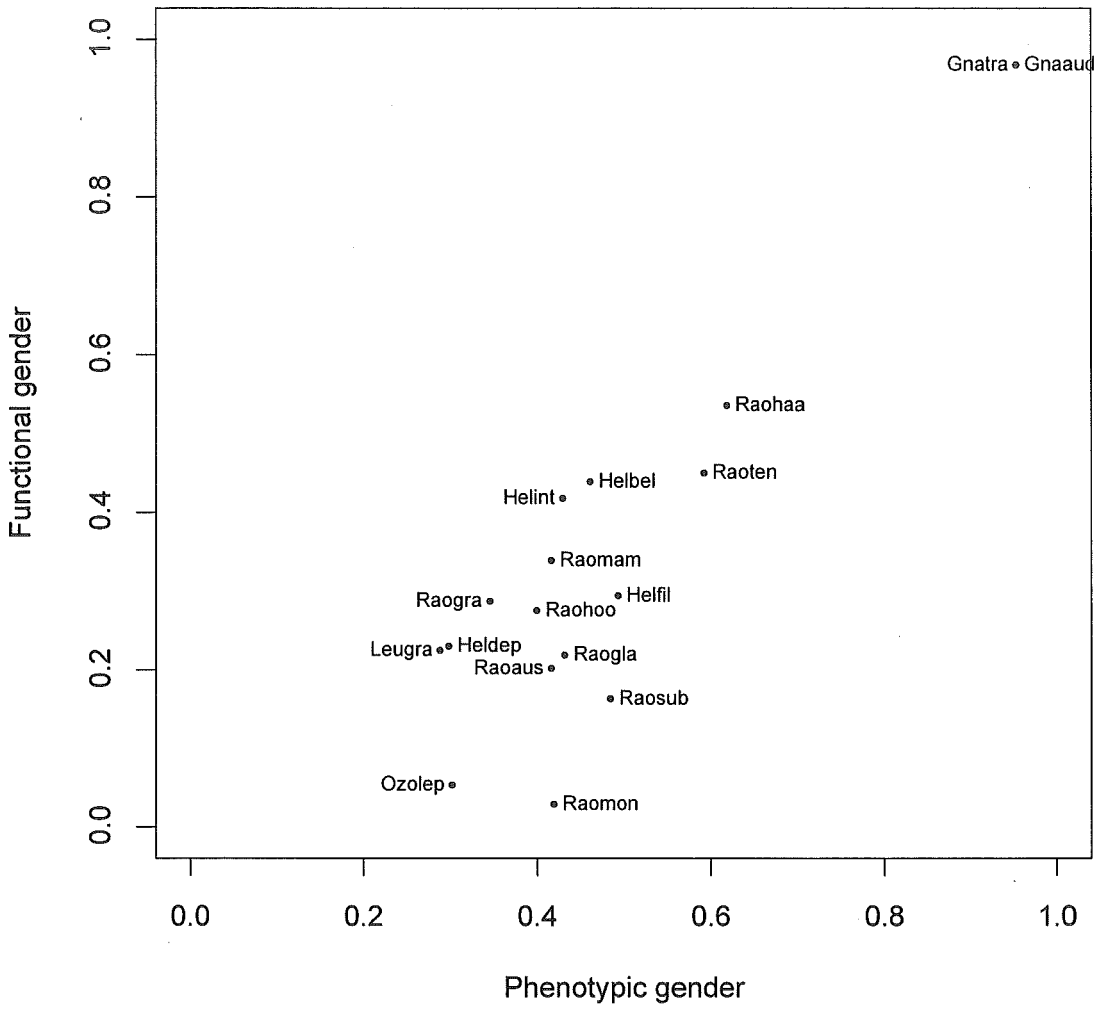


Figure 3.51: The comparative phenotypic and functional gender of 17 species of New Zealand Inuleae.

Floral visitors were observed on capitula of all species except *Gnaphalium audax* and *G. traversii* (Table 3.5). Other species were visited by a range of insect species. Insects were not identified to species level since most were not collected in the field, therefore most floral visitors are only identified to one of nine broad groups (Table 3.5). This excludes the Lepidopteran species, *Lyceana boldenarum* F.B. White (the boulder butterfly), *L. sallustius* Fabricçus (the common copper), *Dasyursis anceps* Butler and *Notorea catapyrrha* cmplx. Butler, all of which were easily identifiable in the field.

Tachinid flies (Plate 28D and E) and Lepidopterans (Plate 28A and B) were the most common floral visitors, both in terms of numbers in the field (pers. obs.) and the number of species which they were observed to visit (Table 3.5). Both these types of insect were observed to feed only from the top of the corolla tube. During foraging the appendages and

mouth parts of these visitors were observed to contact the reproductive floral structures. Pollen grains were also observed occasionally on the abdominal hairs of Tachinid, and other, flies. The Tachinids were observed to spend approximately two to four seconds at each capitulum, while the two butterfly species would remain at each capitulum for up to 15 to 20 seconds.

Dipteran species other than Tachinid flies (Tachninidae) were also common visitors to a range of species (Table 3.5). Hoverflies (Syrphidae) (Plate 28C) were most commonly observed on the capitula of species growing at Broken River, but were also observed on *Ozothamnus leptophyllus* at Cass and Lake Pearson. The hoverflies were the only group of visitors, other than the solitary bees, which were observed to actively take both pollen and nectar. The Tephritid species (Plate 28F) were observed on the capitula of seven species (Table 3.5), and their visits inferred to two other species, *Leucogenes grandiceps* and *Haastia sinclairii,* by the presence of pupae in the capitula. (The identity of the pupae was established by allowing the pupae to hatch in the laboratory.) These flies were observed to use the plants, especially the mat and carpet forming species, as courtship and mating arenas. The courtship and copulation of the flies were frequently observed on the mat forming riverbed species. Oviposition (into capitula) was also observed on a number of instances. Tephritids were observed to visit capitula actively on only a few occasions, feeding at the top of the corolla tube on each of these.

Solitary bees (Apoidae) were also common visitors to a range of species (Table 3.5), although they were observed in greatest numbers on the riverbed species. The bees were observed to collect both nectar and pollen, and were frequently observed with large pollen baskets.

The Hemipteran species, *Nysius* spp. and *Rhypodes* spp (Lygaeidae), were observed on the capitula of species found growing in the proximity of water, mainly being observed on the riverbed species (Table 3.5). Although these species were common visitors to the plants, especially the mat forming species such as *Raoulia australis*, they were observed to visit capitula on only a few occasions. On each of these occasions, they were observed to feed at the top of the corolla tube.

Coleoptera were observed only infrequently, and with the exception of *Ozothamnus leptophyllus*, only on the species at Broken River (Table 3.5). The foraging activity of these species could not be observed accurately as the beetles reacted to even very slight movements by dropping from the capitulum.

Other floral visitors included a range of wasp and ant species (Table 3.5). Both of these type of visitors were observed to feed from the top of the corolla tube. Pollen grains were observed to adhere to the body and antenna of ants on a number of occasions.

The list of visitors to *Helichrysum intermedium* and *R. subsericea* also includes the visitors seen during the night time observations. Both of these species were observed to be visited by moth species at night (at least two species on *H. intermedium*). Collembola were also observed on the capitula of *Helichrysum intermedium* at night. *Gnaphalium audax* and *H. filicaule* were also examined at night, but no visitors were observed to these species.

From the plant species perspective, *Raoulia mammillaris, Helichrysum depressum,* and *H. filicaule* are distinctive. *Raoulia mammillaris* was observed to be visited only by species of fly, and on one occasion by a species of Elateridae (Coleoptera). Visitors to *H. filicaule* were observed on only two occasions; once each by a Tachninid, and also by a small wasp. The main visitors to *H. depressum* were the boulder butterfly, although Tachinid and Tephritid species were also observed infrequently on the capitula.

	boulder butterfly	common copper	moth spp.	other moths	solitary bee	Hemiptera	Coleoptera	Syrphid	Tachinid	Tephritid	Other Diptera	other
Gnaphalium audax												
Gnaphalium traversii												
Haastia sinclairii							+			+*		
Helichrysum bellidioides			+[1]				+ P	+ P	+ P		P	
Helichrysum depressum	+								+	+		
Helichrysum filicaule									+			wasp
Helichrysum intermedium				+ P	+ P	+	P	P	+ P		+	Entomobryidae (Collembola)
Leucogenes grandiceps				+ P	+		+ P	+ P	+ P	+*	+	ants, thrips
Ozothamnus leptophyllus	+	+		+	+ P		+	P	+ P	+	+ P	wasp
Raoulia australis	+		+[2]	+	+	+			+	+	P	
Raoulia glabra	+				+	+	+	+	+		+	
Raoulia grandiflora			+[1]	P	+ P		+ P	+ P	+		+ P	P: Pycnodiamon pluto
Raoulia haastii	+			+	+	+		+	+	+	+	ants
Raoulia hookeri	+ P		+[2]		+ P	+			+ P	+	+	
Raoulia mammillaris									+		+ P	Elateridae (Coleoptera)
Raoulia monroi	+		+[2]	+					+	+	+	ants
Raoulia subsericea	+ P	P	P[2]	+ P	P				+ P	+	+ P	
Raoulia tenuicaulis				+	+	+		+	+		+	ants

Table 3.5: Pollinator groups observed visiting species in the Cass/Craigieburn district between 1994 and 1997 in this study (+) and by Primack (1983) (P). * = inferred by presence of larvae in capitula. [1] = *Dasyuris anceps*. [2] = *Notorea catapyrrha* cmplx.

Plate 17: Study sites

A: The Cass Fan and Field station, with Sugarloaf in the background.

B: The study site at the Cass river.

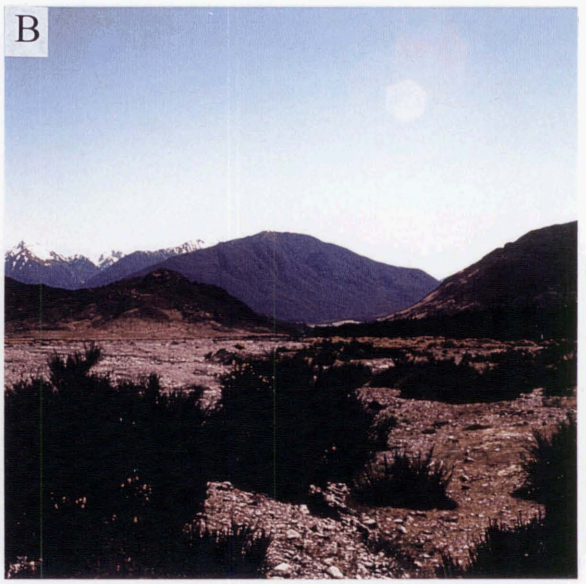
C: The study site at Broad Stream (arrowed).

D: The bluff system at Dry Stream, with the edge of the old river terrace visible to the lower right.

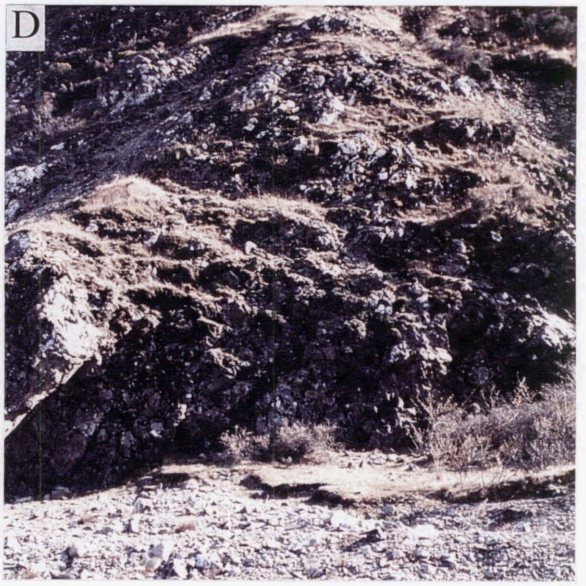
E: The late snow hollow in Allan's Basin. Arrow indicates the habitat of *Raoulia subulata.*

F: The (old) glasshouse at Canterbury University.


A


B


C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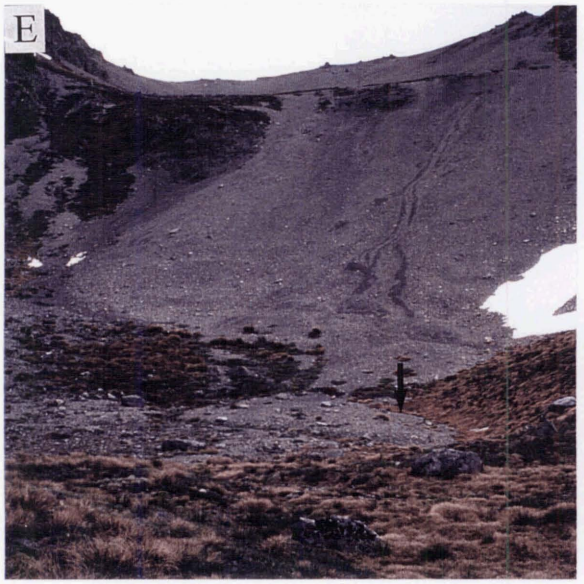

E


F

Plate 18: Broken River Ski Field and Allan's Basin, Craigieburn Range.

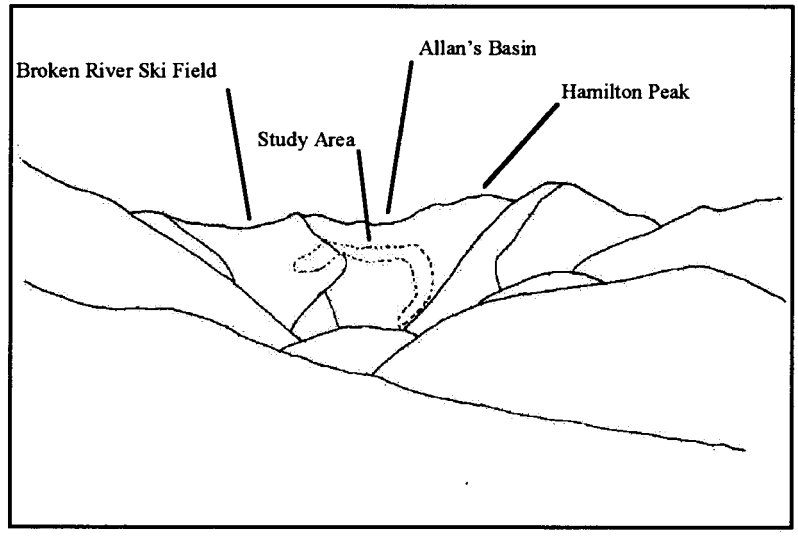
The pattern of snow melt recorded on

A: 3 October 1996

B: 28 November 1996

C: 6 January 1997

D: 21 January 1997.



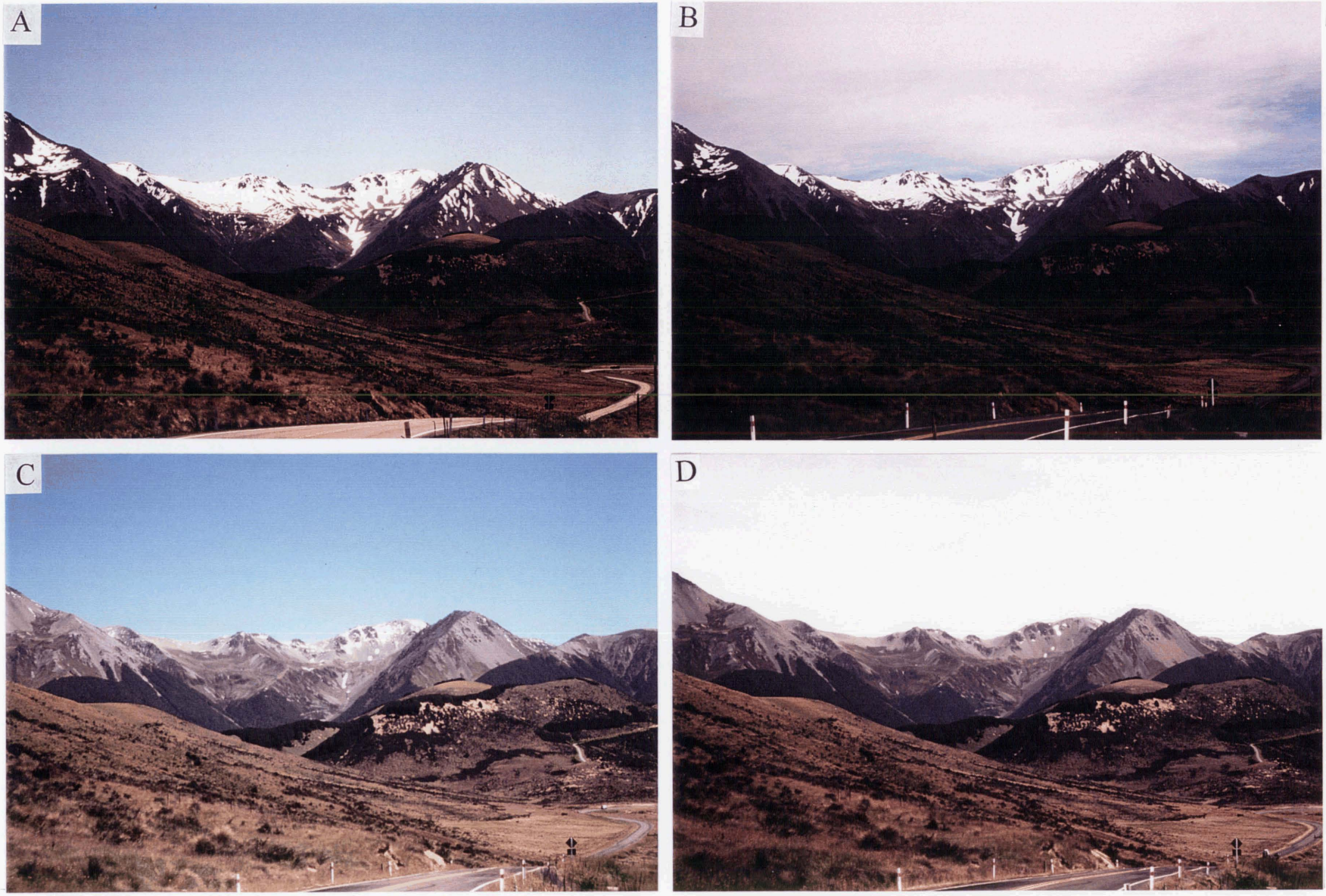

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Plate 19: Stage in the capitula phenology of *Gnaphalium traversii*, *Leucogenes grandiceps*, and *Raoulia glabra*.

A: Capitulum of *Gnaphalium traversii* at early anthesis showing the tips of the filiform style arms extending between the involucral bracts. (*c.* 40- 45 times life size).

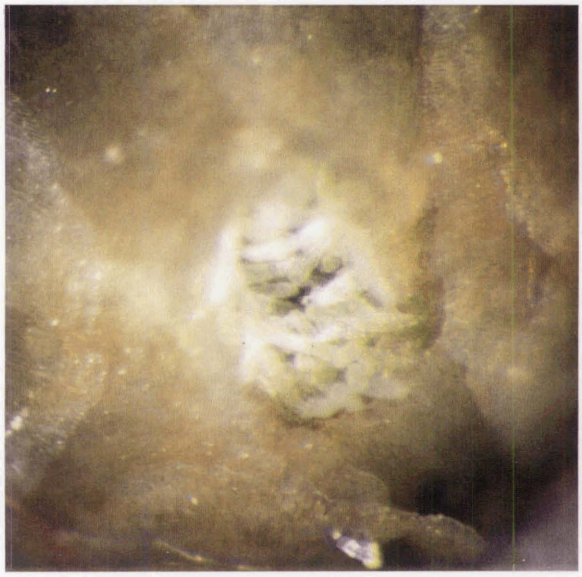
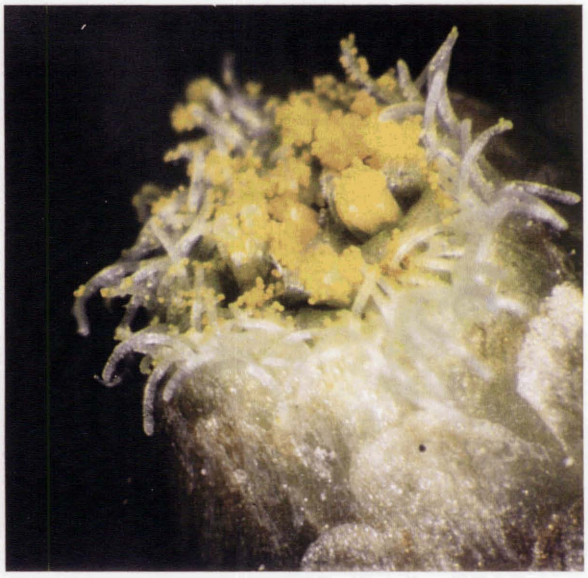
B: Capitulum of *G. traversii* at early anthesis showing partially extended filiform styles, and a tubular floret presenting pollen. (*c.* 35 times life size).

C: Flowering head of *Leucogenes grandiceps* showing the central capitulum with all filiform and three tubular florets at anthesis. (*c.* 6 times life size).

D: Flowering head of *L. grandiceps* showing a range of stages of anthesis in the capitula. (*c.* 4 times life size).

E: A bud and capitulum at early anthesis of *Raoulia glabra.* (*c.* 6 times life size).

F: The cup shaped corolla of the tubular florets of *Raoulia glabra*, during the male phase of anthesis. (*c.* 25 times life size).

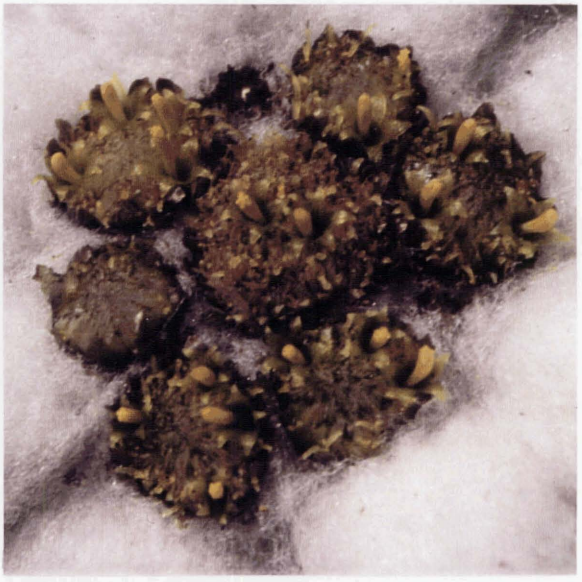


Plate 20: Stages in the capitula phenology of *Helichrysum bellidioides*.

A: Capitulum at start of anthesis with filiform styles visible between the pappus. (*c.* 35 times life size).

B: Capitulum in early anthesis with first of tubular florets opening. (*c.* 35 times life size).

C: Capitulum in early stages of anthesis, with early tubular and filiform florets presenting styles. (*c.* 35 times life size).

D: Capitulum in early stages of anthesis. (*c.* 13 times life size).

E: Capitulum at late anthesis, with nearly all florets open. (*c.* 10 times life size).

F: Capitulum immediately following anthesis with all florets brown. (*c.* 10 times life size).

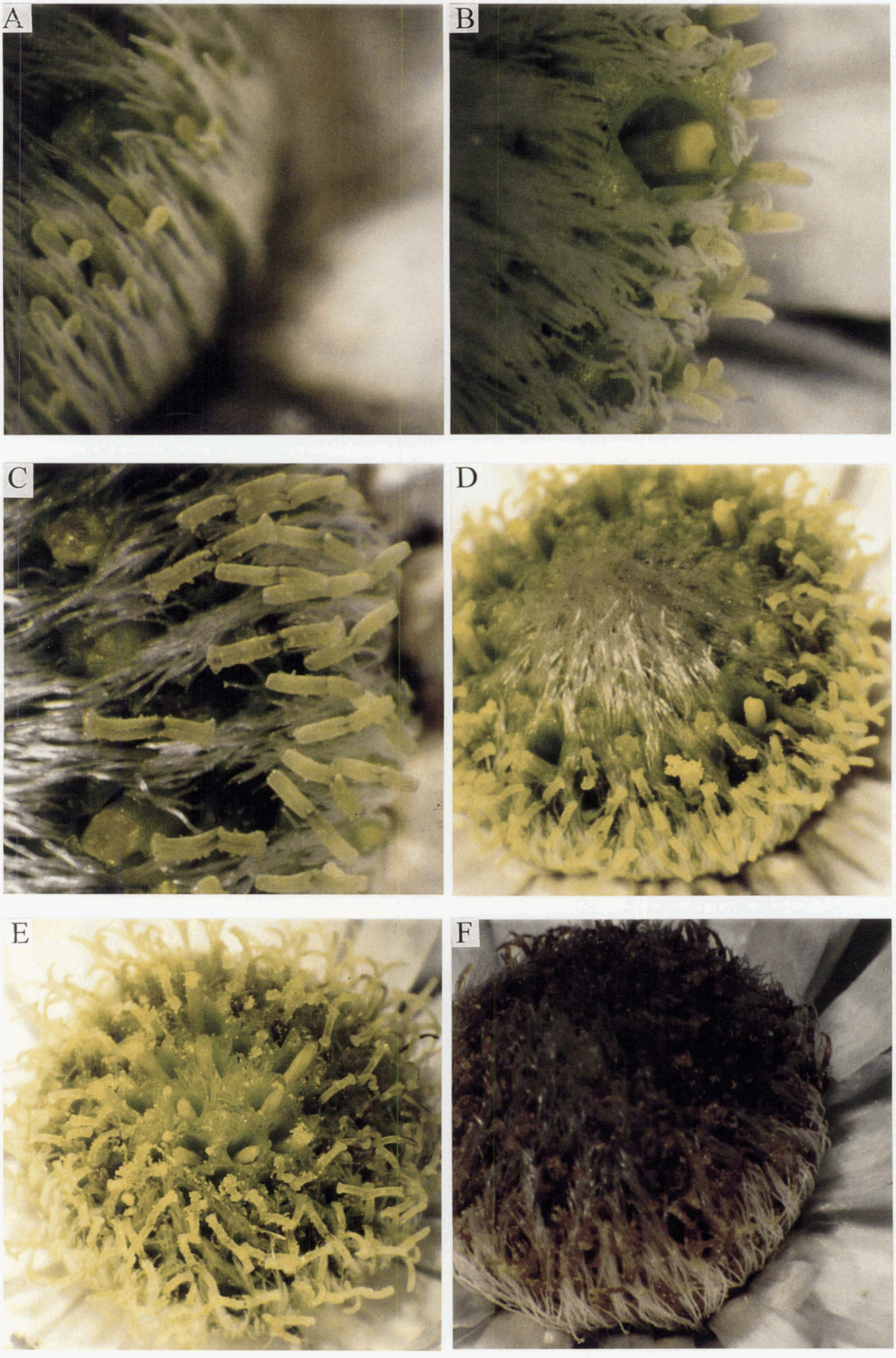

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Plate 21: Stages of capitula phenology in *Helichrysum filicaule*

A: Capitulum in early stages of anthesis with filiform florets presenting styles (arrowed). (*c.* 15 times life size).

B: Capitulum with filiform florets presenting styles and the first tubular florets presenting pollen. (*c.* 15 times life size).

C: Capitulum in early stages of anthesis. Note the florets at anthesis beginning to bend. (*c.* 10 times life size).

D: Capitulum at mid to late anthesis with nearly all florets open. Note the curled style arms. (*c.* 12 times life size).

E: Capitulum with all florets open. Outer florets beginning to brown. (*c.* 12 times life size).

F: Capitulum in late anthesis with all florets brown or in female phase. (*c.* 12 times life size).

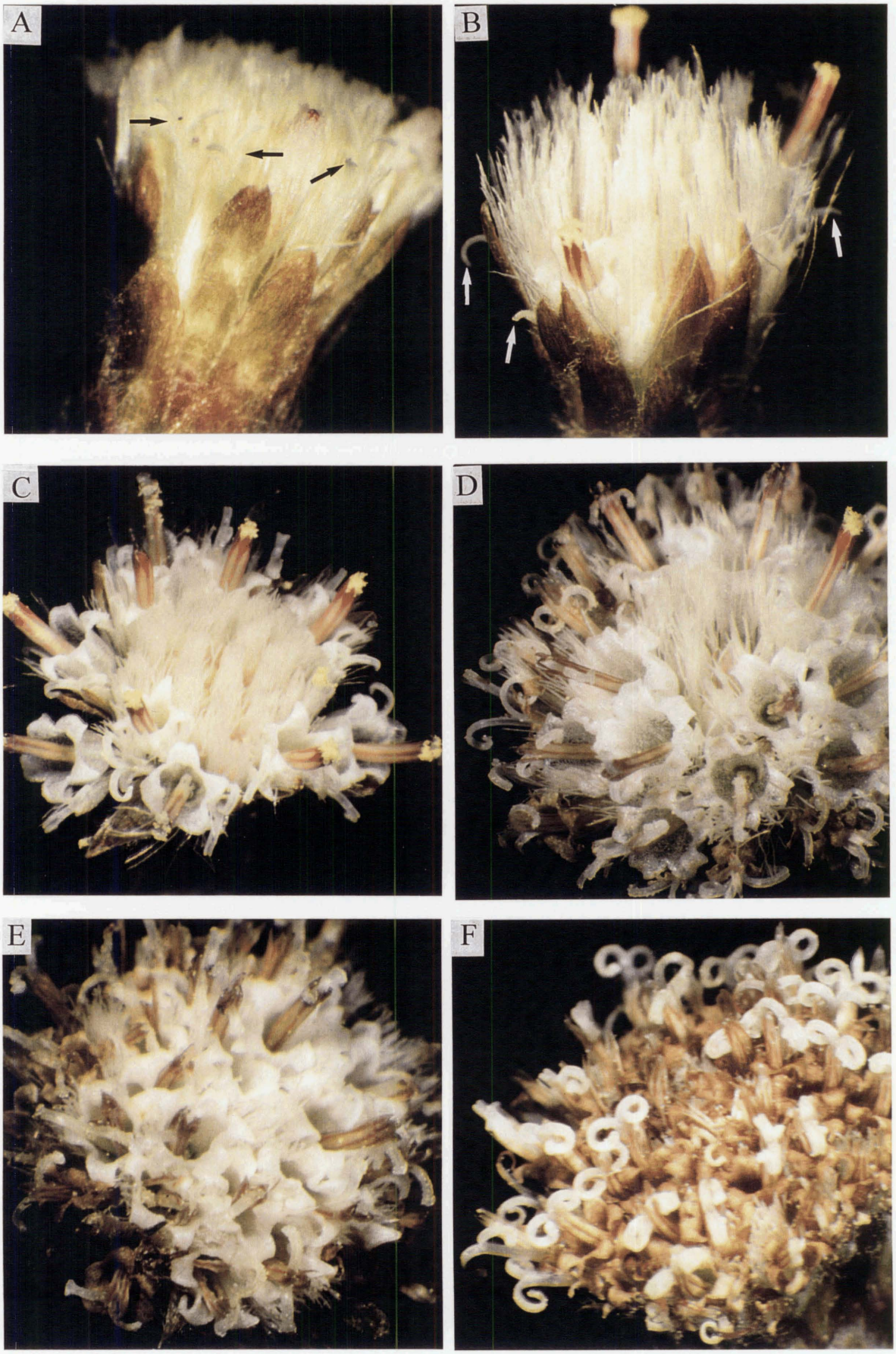
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Plate 22: Stages in the capitula phenology of *Helichrysum depressum* and *Raoulia subsericea*.

A: Capitulum of *Helichrysum depressum* in early anthesis, showing four recently emerged tubular florets. (*c.* 15 times life size).

B: Capitulum of *Helichrysum depressum* in late anthesis, showing the last tubular floret just prior to pollen presentation. (*c.* 20 times life size).

C: Capitulum of *Helichrysum depressum* which has completed anthesis. (*c.* 18 times life size).

D: Capitulum of *Raoulia subsericea* showing two tubular florets emerging (short arrows) and another presenting pollen (long arrow). (*c.* 20 times life size).

E: Capitulum of *Raoulia subsericea* showing the first tubular floret in female phase (long arrow) and subsequent tubular florets presenting pollen (short arrows). Note well spread filiform styles. (*c.* 18 times life size).

F: Capitulum of *Raoulia subsericea* with all florets open. Note style tips beginning to brown. (*c.* 14 times life size).

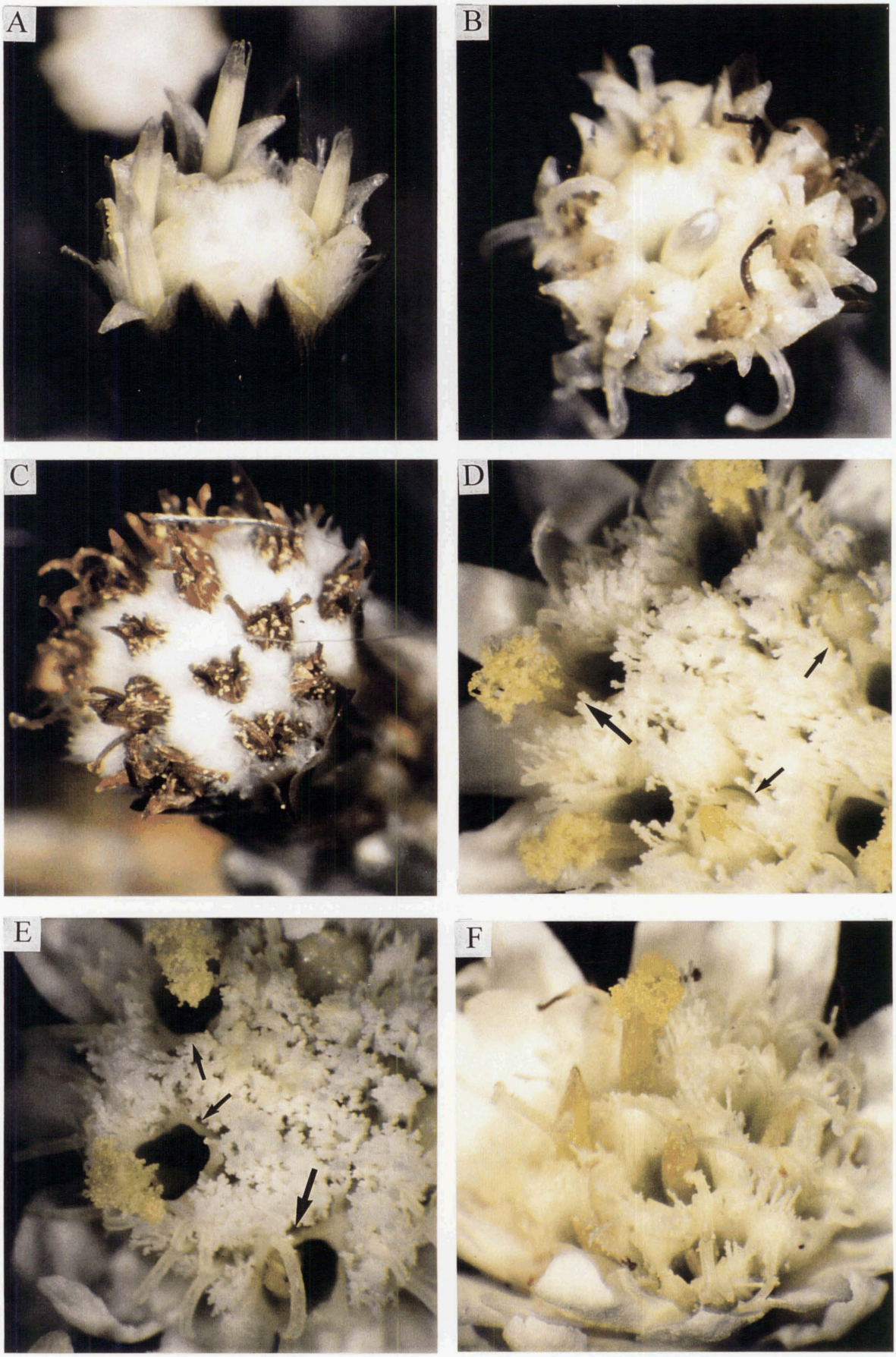
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Plate 23: Stages of capitula phenology in *Helichrysum intermedium* and *Raoulia monroi.*

A: Capitulum of *Helichrysum intermedium* with four tubular florets at anthesis (arrowed). (*c.* 8 times life size).

B: Capitulum of *Helichrysum intermedium*, showing tubular florets beginning to bend. (*c.* 14 times life size).

C: Capitulum of *Helichrysum intermedium* at the end of anthesis with all styles brown. Note the rounded appearance of the capitulum. (*c.* 10 times life size).

D: Capitulum of *Raoulia monroi* with filiform florets presenting styles. Note tubular florets emerging between pappus. (*c.* 18 times life size).

E: Capitulum of *Raoulia monroi* at mid anthesis with tubular florets at a variety of stages. (*c.* 18 times life size).

F: Capitulum of *Raoulia monroi* with all florets presenting styles. (*c.* 18 times life size).

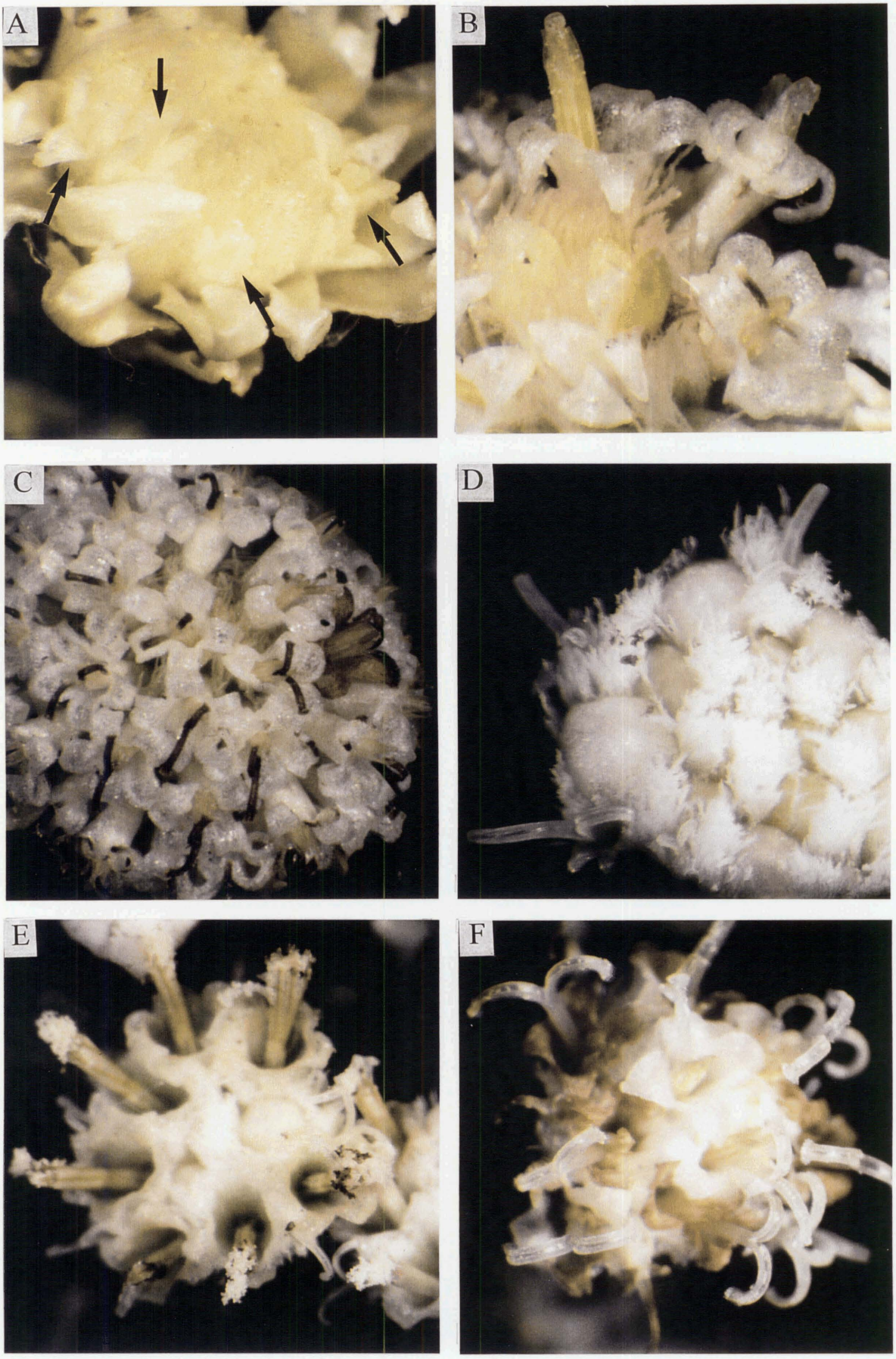
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Plate 24: Stages in the capitula and floret phenology of *Ozothamnus leptophyllus*.

A: Buds at varying stages of development. (*c.* 10 times life size).

B: A tubular floret showing the swollen corolla immediately prior to the corolla opening. (*c.* 40 times life size).

C: Capitula in the early stages of anthesis, with one tubular floret presenting pollen and the top of another emerging between the bract and pappus. (*c.* 8 times life size).

D: Capitulum with a tubular floret in early female phase. (*c.* 30 times life size).

E: Capitulum in early to mid-anthesis showing florets in a range of stages. (*c.* 30 times life size).

F: A capitulum near the end of anthesis, showing the last tubular floret opening and the curled styles of the other florets. (*c.* 25 times life size).

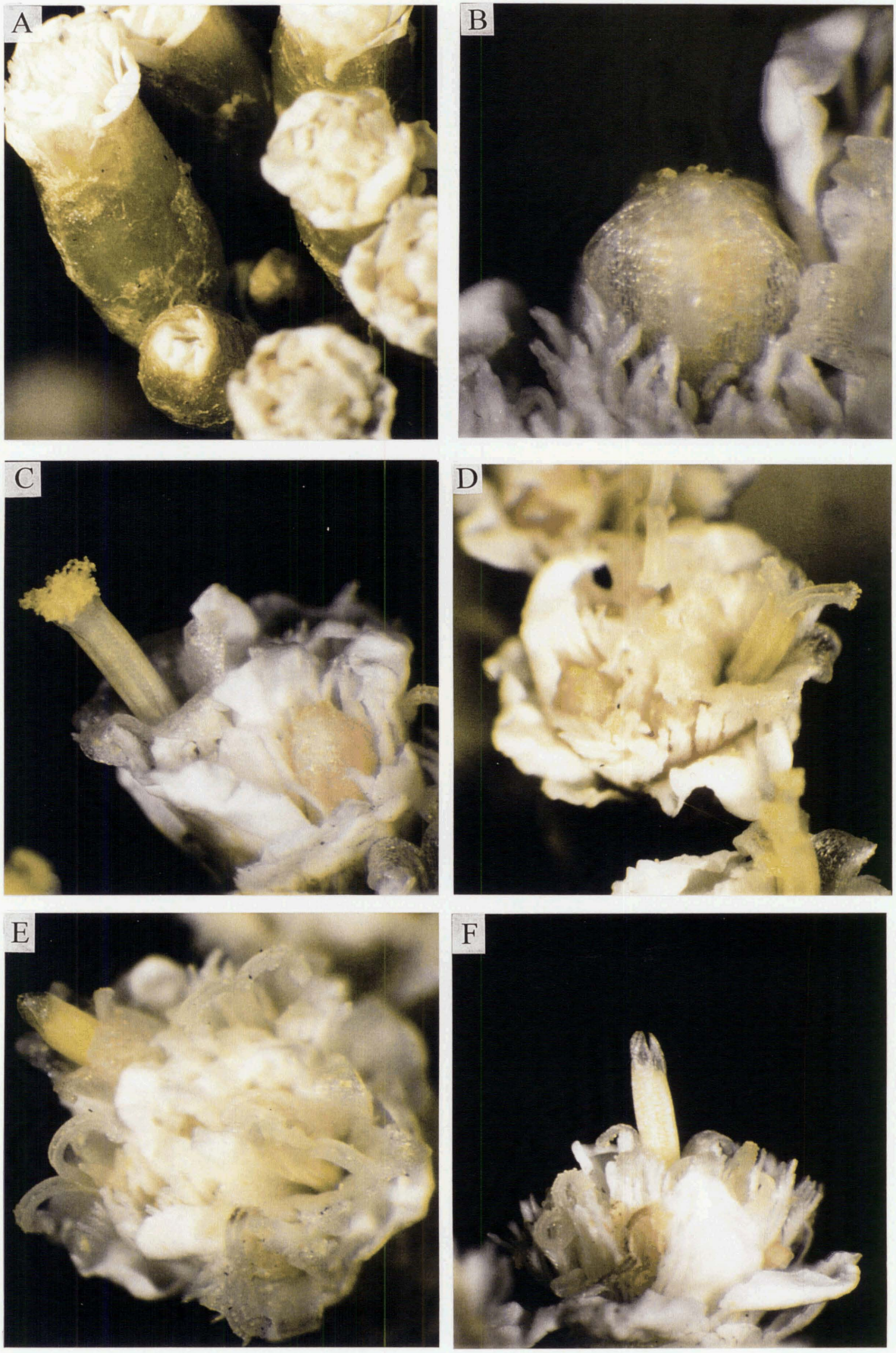
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Plate 25: Stages in the capitula phenology of *Raoulia australis*.

A: Capitulum in early anthesis, with filiform florets presenting styles (arrowed). (*c.* 50 times life size).

B: Capitulum at mid-anthesis. Filiform and most tubular florets in female phase, one tubular presenting pollen (arrow). (*c.* 50 times life size).

C: Capitulum in the early stages of anthesis, showing the first tubular florets open. (*c.* 50 times life size).

D: Capitulum in late anthesis, with all florets presenting styles (some tubular styles separated). (*c.* 50 times life size).

E: Capitula at the end of anthesis, with the styles beginning to brown. (*c.* 50 times life size).

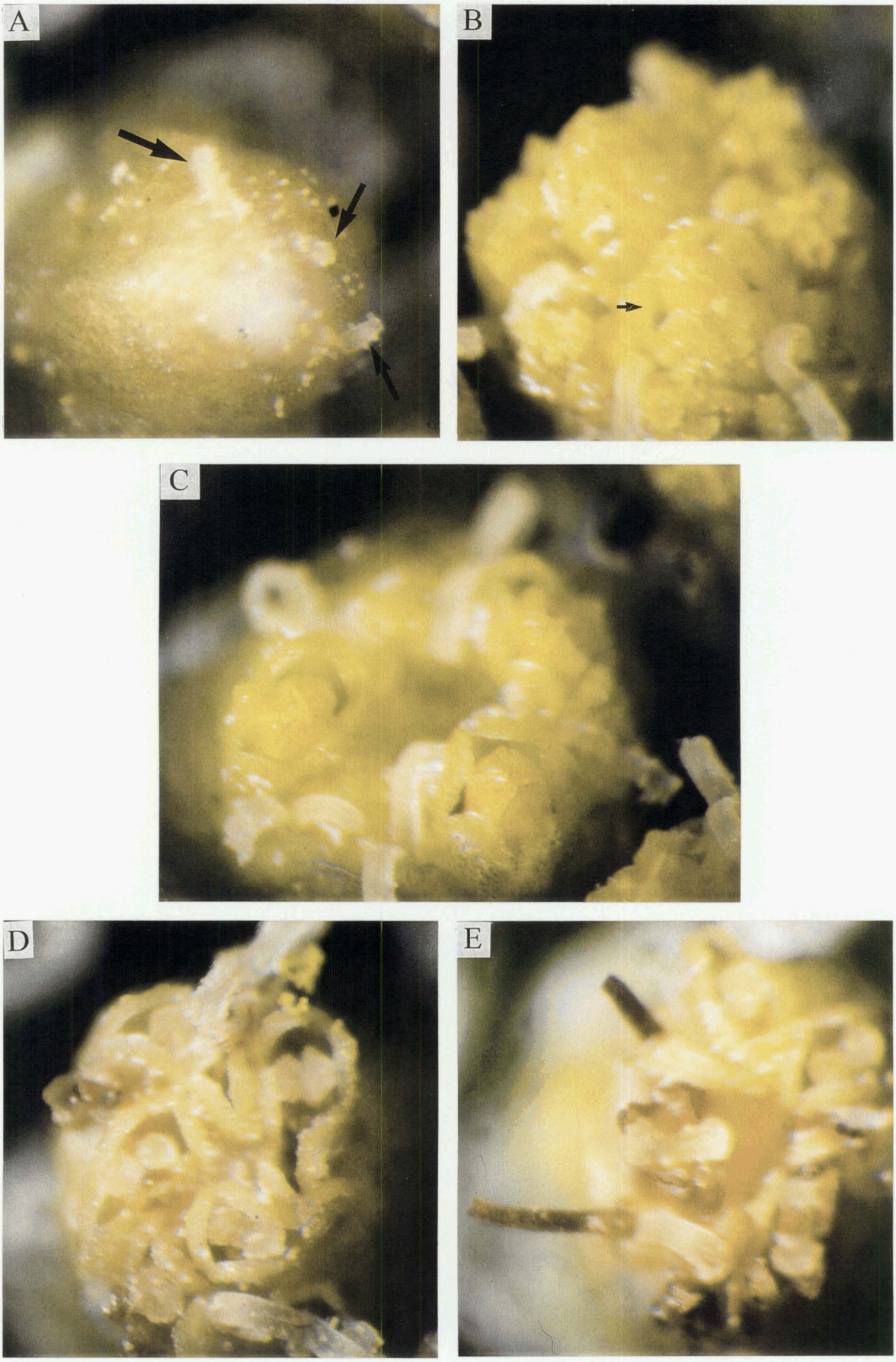
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Plate 26: Stages in the capitula and floret phenology of *Raoulia mammillaris* and *Raoulia grandiflora*

A: Capitulum of *Raoulia mammillaris* in early anthesis, with the first tubular florets beginning to open. (*c.* 24 times life size).

B: Capitulum of *R. mammillaris* in early anthesis; One tubular floret visible in early female phase, another just about to begin pollen presentation. (*c.* 26 times life size).

C: Two capitula of *R. mammillaris*. The capitulum to the left, in early anthesis; two tubular florets in early female phase, another about to begin pollen presentation. Capitulum to the right about to begin anthesis. (*c.* 18 times life size).

D: Capitulum of *Raoulia grandiflora* showing two recently opened tubular florets, and the filiform styles at the edge of the pappus. (*c.* 18 times life size).

E: Capitulum of *Raoulia grandiflora* showing filiform styles extended above the pappus, and a tubular floret just about to begin pollen presentation. (*c.* 20 times life size).

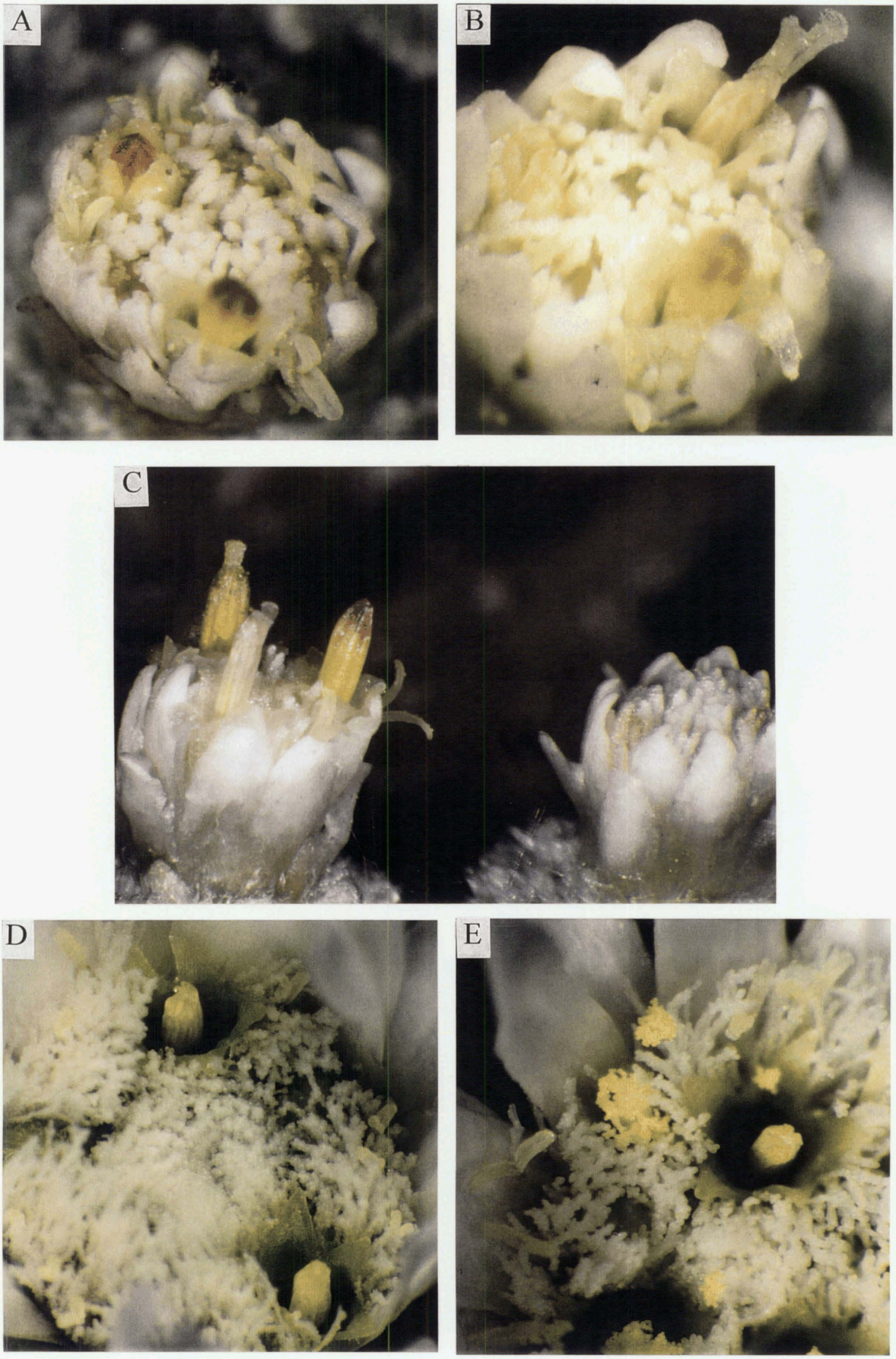
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Plate 27: Stages in the capitula and floret phenology of *Raoulia haastii.*

A: Two capitula in early anthesis, both with a single, recently opened tubular floret presenting pollen. (*c.*25 times life size).

B: Capitulum of *R. haastii*, showing two tubular florets at anthesis. The left and right florets are in early female phase and male phase respectively. (*c.*60 times life size).

C: Four capitula of *Raoulia haastii*, showing two capitula with all florets in female phase (bottom left, and top right), one capitulum with one tubular and the filiform florets at anthesis (top left), and the fourth capitulum with all florets open, one tubular floret presenting pollen. (*c.*20 times life size).

D: Close up of a capitulum of *R. haastii* with all florets in female phase. (*c.*60 times life size).

E: Two capitula of *R. haastii* in late anthesis. A short stamen tube is visible in the right capitulum. (*c.*30 times life size).

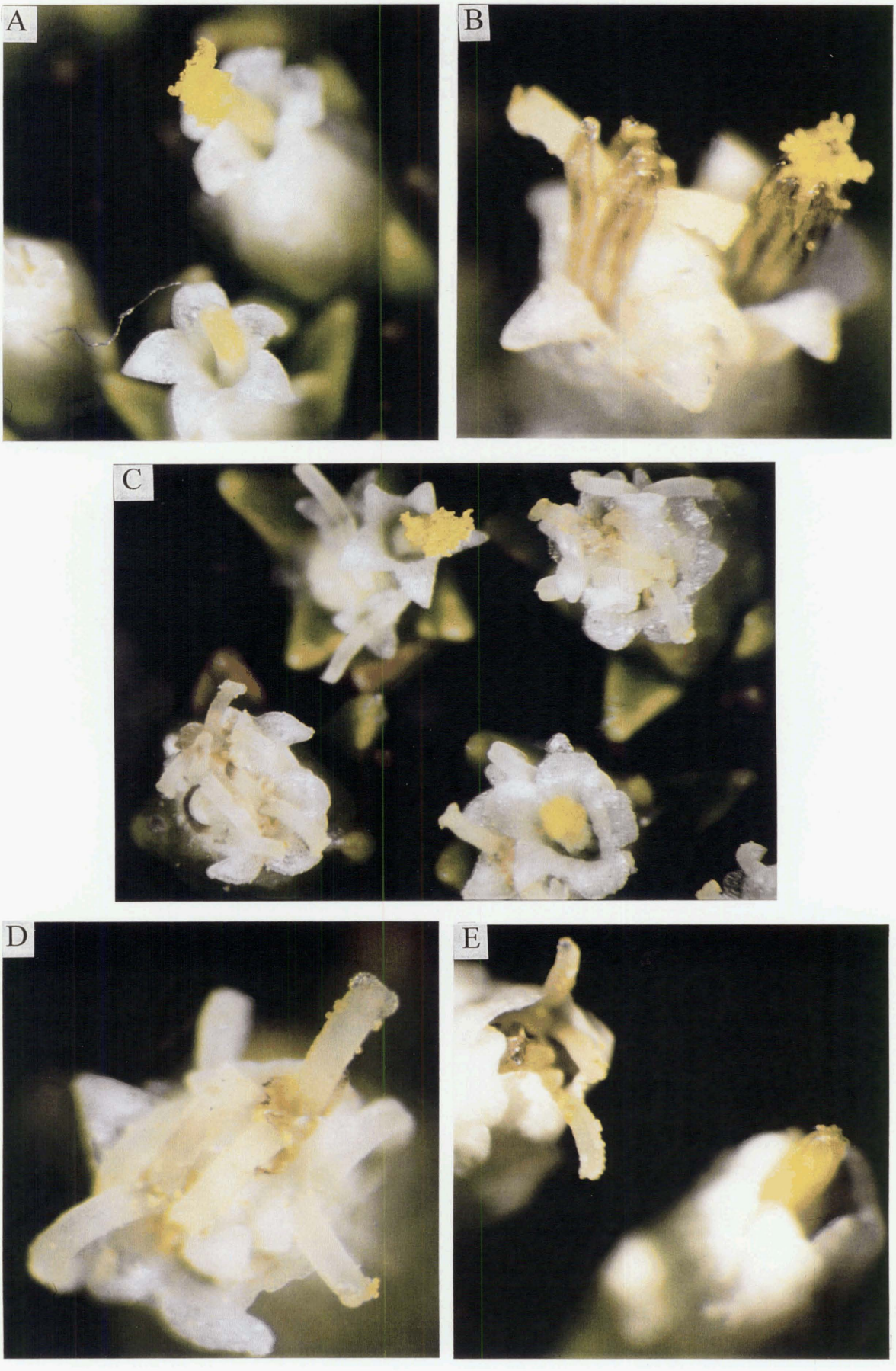
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Plate 28: Insect visitors.

A: *Lyceana boulderanum* on a capitulum of *Raoulia glabra*. Note the proboscis probing the floret. (*c.* 4 times life size)

B: *Lyceanea bouderanum* feeding from a capitulum of *Raoulia haastii*. (*c.* 3 times life size)

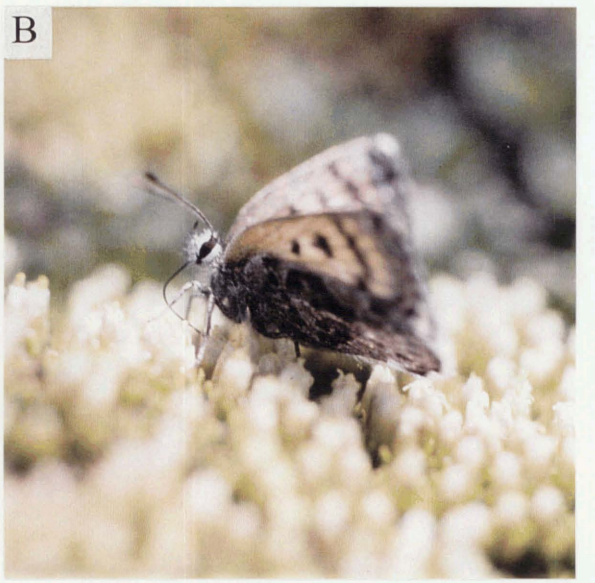
C: A hoverfly (Syrphidae) visiting a capitulum of *Leucogenes grandiceps* x *Helichrysum bellidioides*. (*c.* 3 times life size)

D: A Tachinid fly visiting a capitulum of *Raoulia glabra*. (*c.* 4 times life size)

E: A Tachinid fly on a mat of *Raoulia tenuicaulis*. (*c.* 4 times life size)

F: A Tephritid fly on *Raoulia haastii*. (*c.* 4 times life size)


A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3.5 Discussion

Population and Association Patterns

The effect of altitude and climate on the flowering phenologies is clearly demonstrated by the occurrence of the same species in different associations. The delays in flowering season of two to three weeks at Broken River compared to the starting dates of species at the lower sites are consistent with the delays of reported in other studies (e.g. Scott, 1966; Clarke, 1968). The delay of the start of flowering at Dry Stream, when compared to Broad Stream, may be partially explained by the greater shading that occurs at this site, compared to the other sites, due to the narrow and steep side topography of the Dry Stream valley. Webb (1976) found that *Gingidia decipiens* growing on a shaded slope flowered 10 days later than plants on a neighbouring sunny slope. The differences of starting dates between Broad Stream and the Cass River are less easily explained, but may result from the accumulation of cold air in the Cass Basin (Greenland, 1977). The site on the Cass River sits in the valley bottom and has only a shallow slope, while the Broad Stream site is on a river fan approximately 80 m above the bottom of the main valley, with a steeper slope, thus the cold air would be able to drain away. It is notable that despite these differences in the starting times of individual species among the sites, there is a consistent sequence of the flowering among the species within each site. This preservation of the order in the flowering sequence has also been found by Arroyo (1990) and Ghazanfar (1997).

The influence of climate is also apparent when the phenological patterns are compared between associations. The most apparent contrast is that of the alpine association. The duration of the flowering time of the individual species, and of the species when combined, is shorter than the flowering times observed in the riverbed and grassland associations. This phenomenon has been well documented in other species (e.g. Scott, 1966; Clarke, 1968), and has been attributed to the shorter growing season in the alpine environment, particularly the length of the snow-free period. In the harsh alpine environment any individual that begins flowering too early or too late can be expected to suffer a high degree of abortion (e.g. Webb, 1976; Kudo, 1993), or pollen and/or resource limitation (e.g. Totland, 1997). The length of the snow-free period also explains the different length of flowering time in *Raoulia subulata*, a specialist late-snow hollow species, in the 1994/95 and 1995/96 season. In the 1993/94 season snow melt occurred much more slowly than the following year, restricting the flowering of the population to only a few weeks; by contrast

the quicker snow-melt in the 1995/96 season allowed the same plants to flower for over 6 weeks (pers. obs.). The apparently extended season of *Haastia* is also correlated with snow melt patterns, with a number of *Haastia* individuals growing in an area which was consistently one of the last slopes to become snow-free each year (pers. obs.). The delay in flowering in the individuals which grow in areas covered by late melting snow appears to be a common feature of the alpine environment (e.g. Spence, 1989; Kudo, 1993; Stanton *et al.*, 1997; Wagner and Reichegger, 1997).

Climatic influences are also apparent in the phenological patterns of the grassland species, most notably the duration of the flowering season of *Gnaphalium traversii*. The population of this species flowered for only 4 weeks during the 1994/95 season, compared with 8 and 10 weeks in the 1993/94 and 1995/96 seasons. This difference is probably the result of low soil moisture levels prior to and during flowering in the 1994/95 season. This is suggested by the much lower rainfall during July to November of the 1994/95 season (Figure 3.6). The higher rainfall during January of the 1994/95 season occurred after the population had finished flowering, and would therefore be ineffectual in prolonging the flowering season.

Another feature which is apparent when the phenological patterns of each association are compared is the greater degree of synchrony between individuals in the riverbed and alpine associations. In both these associations most individuals began flowering at about the same time. In the alpine association this may result from the shorter season providing less opportunity for variation, but the synchrony of riverbed species cannot be explained by this, since overall season length is long (nine months). However it may be explained by selection for discrete flowering periods (see below).

The most prominent difference among associations is the staggered flowering pattern of the riverbed species. Two hypotheses, that are not mutually exclusive, may explain the selective processes that may have resulted in this flowering pattern.

The braided river systems are subject to occasional large floods, which result in major changes in channel location and loss of old river terraces. The probability of flows exceeding two or three times the mean flows are not evenly distributed throughout the year, with the greatest probability of high river flows (in the Waimakariri Catchment at

least) occurring during November (Figure 3.5). Given the destructive power of these periods of high water and the uneven annual distribution of river flows, these high flows potentially represent a considerable selective force upon the riverbed species. That this selection pressure could result in different flowering times among the study species is dependent on the fact that the species have different ecological distributions. Foweraker (1917) divided riverbed into three stages of stability, from the most recently disturbed riverbed, with very sparse or no vegetation, through to the old river terraces, with communities of *Discaria toumatou* and a variety of grasses. In the middle stage he recognised three grades, with each successive grade having less chance of being flooded and greater stability. Foweraker found that each of the riverbed species had a particular grade upon which it was most common. *Raoulia tenuicaulis* was most common on the lower grades occurring on most recently disturbed areas and near stream margins. *R. hookeri* occurred on the lower grades, but on more stable grades than *R. tenuicaulis*. In contrast *R. haastii* and *R. australis* occurred on the most stable grades. When the flowering times of these species are compared to their distribution on riverbed, and the probability of flooding, a striking pattern emerges. *R. tenuicaulis*, which grows in most disturbed areas, and is therefore likely to have capitula damaged or washed away by floods, began flowering before the peak in the probability of being flooded. By contrast, *R. haastii*, which flowers during the period which has highest probability of flooding, grows on the most stable grades where it is unlikely to be flooded. Similarly *R. australis*, which flowers when the probability of flooding is still high, also occurs on stable grades. *R. hookeri*, which is the most vulnerable species after *R. tenuicaulis*, begins flowering after the highest probability of a flood occurring. Thus selection may have favoured early flowering individuals, which are able to release seed before floods in *R. tenuicaulis*, while in *R. hookeri*, on the slightly more stable riverbed, selection appears to have favoured later flowering, possibly due to higher numbers of pollinators or an ability to accumulate greater reserves prior to flowering. The ability of *R. hookeri* to survive flooding and then flower in the same season, was observed in 1993/94 when floods in early 1993/94 covered many of the tagged plants of *R. hookeri* with a layer of silt, yet these individuals still flowered in that season. It is also notable that *R. tenuicaulis* and *R. haastii* both flower earlier than *R. hookeri* and *R. australis* and also possess smaller capitula, indicating that early flowering in these species may have been allowed due to fewer resource requirements or short developmental times. No information is available to test this hypothesis.

This hypothesis fails to explain fully the staggered flowering times, particularly of *R. australis*, *R. haastii* and *Helichrysum depressum*, all of which occur on the more stable grades and could therefore potentially flower at any time with minimal chance of flooding. These staggered flowering times could, however, be explained by pollination competition.

Competition for pollinators has been implicated in selection for staggered flowering time in a number of communities where plant species share pollinators (e.g. Hurlbert, 1970; Stiles, 1977). This can occur through direct competition for the pollinator service (e.g. Gross and Werner, 1983), or through the loss of fitness in individuals due to interspecific pollinations resulting in pollen wasting and stigma clogging (e.g. Waser, 1978b; Campbell and Motten, 1985). Interspecific pollen transfer may also result in hybrid offspring which would compete for establishment sites and pollinator services in subsequent years. Given the wide range and relatively high density of insect visitors observed on these species, it is unlikely that direct competition for services of pollinators would occur in these species. However, the interspecific transfer of pollen may be a strong selective pressure given the generalised structure of the capitula, the close proximity of the species, and the relatively high incidence of hybridisation in the New Zealand Inuleae (e.g. Allan, 1961). (*R. australis* plants 1 and 4 at Dry Stream (Figure 3.19) are potentially hybrid offspring. Although morphologically indistinct from the few individuals of *Raoulia australis* at this site, these individuals flower at the same time as the *R. hookeri* population.) It is therefore hypothesised that the combined effects of pollinator competition and the distribution of flooding has resulted in a staggered flowering time. The overlapping flowering of *R. hookeri* and *Helichrysum depressum* may not have been selected against, as *H. depressum* was observed to be mainly visited by butterflies. This specific pollinator relationship may be sufficient to reduce competition between these species.

If strong selection pressures are influencing the phenology of species, this must occur through selection at the individual level such that early and later flowering individuals in a population would be at a disadvantage. As a consequence of this, the individuals in such populations should be expected to show a high degree of synchrony. This phenomenon was observed in the riverbed species. The individuals of the two species in the middle of the flowering pattern, *R. australis* and *R. haastii*, showed a high level of synchrony both at the start and end of anthesis, while *R. hookeri* and *H. depressum* show a high degree of

synchrony at the start of the season, but are poorly synchronised at end of the season. This pattern would be expected if there were no strong selective pressure to end flowering, but strong competition from early flowering species occurred.

By comparison to the riverbed species, the flowering phenologies of the species in the grassland association overlap considerably. Given that these grassland species were also observed to be visited by a wide range of insect species and grow in close proximity, the lack of staggered flowering times appears to contradict the hypothesis of pollination competition. However, the riverbed and grassland habitats differ in two key aspects, the composition of the vegetation and the density of vegetation. In the riverbed the *Raoulia* species are one of, if not, the major component of the flora (pers. obs.). By comparison, in the grassland vegetation, the study species are only a small component of the flora. Over 40 indigenous dicotyledon species are listed as occurring in grassland by the checklist of flora in Burrows (1977). Of these, approximately one quarter are members of the Compositae. The grassland vegetation is very dense, with only a few very small areas without vegetation cover, compared to the riverbed where most of the substrate is not vegetated, especially in the lower grades. The dense and more varied composition in grassland may mean that direct or interference competition with other species which are not congeneric (diffuse competition) may be more intense than competition for pollinators between the study species. Diffuse competition has been found in other Compositae, for example Gross and Werner (1983) found lower seed set in early flowering individuals in *Solidago graminifolia* due to competition for pollinator service with other grassland species. The higher levels of seed set in late individuals of *Solidago graminifolia* occurred despite the flowering period of late individuals overlapping with two other congeneric species (Gross and Werner, 1983). An alternative hypothesis to explain the lack of staggering is that the flowering time of grassland species is not optimal for flowering, but rather represents a balance between timing of flowering and seed release, as suggested by Rathcke and Lacey (1985) and Fagerström and Ågren (1980). Later seed release may be favoured as the seed would not be required to survive or germinate during February/March, the driest months at Cass (Greenland, 1977). Given that there appears to be no delay in seed release once they are mature and that the seed will germinate immediately following release under glasshouse conditions, the release of seed in late summer/autumn may be favoured, and therefore potentially restrict flowering to later in the season. Unfortunately, no data is available on germination times or seed survival in natural conditions in these

genera, athough seeds of *Chrysothamnus* and *Artemisia*, which also flower in late summer and early autumn, have been found to germinate in very cold conditions in their natural habitat (Young and Mayeux, 1996).

That selection may not be acting strongly (or directly) on flowering time via pollinator competition in the grassland association is also suggested by the poor synchrony between individuals. However, this may also be explained by disruptive selection by seed predation, especially in *R. subsericea*, where a very high proportion of seeds are subject to predation (pers. obs.). English-Loeb and Karban (1992) found that highly synchronised individuals which flowered during the peak flowering period of the population suffered more predation than early or poorly synchronised flowering individuals.

These hypotheses could be tested empirically using plants grown in the glasshouse which flower earlier, allowing them to be placed in the field, establishing the pattern of seed predation across the flowering season, detailed analysis of the plant-plant and plant-pollinator interactions in the grassland, and trials of seed germination and survival.

A second phenological phenomenon observed only in the riverbed association was the occurrence of a gap between the end of flowering in *Raoulia haastii* and start of flowering in *R. australis*. In other studies, such gaps have been found to correlate with diapause or other life cycle stages in the pollinator, which result in a paucity or absence of visitors during this period (Newstrom *et al.*, 1994). However, given the varied nature of the pollinating fauna in this study, it is unlikely that such correlations exist in these species. Pollinator competition also fails to explain this gap, since an absence of a competitor for the pollinator service would favour either early or late individuals of the species on either side of the gap, such that the gap would be maintained. An alternative hypothesis is that occasional competition from a third species may be sufficient to create the flowering gap. A likely candidate for this third species is *R. monroi* for the following reasons: (1) The flowering of this species coincides with the gap between *R. haastii* and *R. australis*. (2) *R. monroi* occurs occasionally on the riverbed but is also common in the open vegetation which occurs on the old river terraces adjacent to the open riverbed in unmodified systems. (3) *R. monroi* is visited by the same insect groups as riverbed species. Observations of the few plants located on the riverbed at Cass showed high insect visitation rates, especially by butterfly and moth species. Unfortunately, empirical data on

the frequency with which *R. monroi* occurs on riverbed, and on the movement of insect species between vegetation types, which would be needed to verify this hypothesis, are unavailable.

Individual Patterns

While the fortnightly sampling interval is sufficient to draw conclusions at the population and association levels, care must be taken when interpreting the phenological patterns at the individual level. For example, a two week interval is insufficient to show accurately the distribution of peaks in flowering of a single individual, or the finer pattern of synchrony between individuals. Despite these limitations, the data indicate three main trends at the individual level.

An important trend related to synchrony of individuals is the consistent order of first flowering date in populations with non-synchronous individuals (e.g. *R. subsericea*). While this could possibly be explained by micro-site differences, a consistent order of anthesis also occurred in successive years in genets grown in the glasshouse (pers ob.). The same type of phenological pattern has been attributed to the influence of genotype in other studies (e.g. Stratton, 1991; Tarasjev, 1997).

The second trend is the occurrence of non-flowering individuals. This appears to be most common in the grassland (e.g. *Helichrysum filicaule*) and alpine (e.g. *Raoulia grandiflora*) species, but also occurs to a lesser extent in the riverbed species (most notably *H. depressum* at Cass). On the riverbed, with its potentially lower levels of survival due to flooding, individuals that flowered each year would potentially have a selective advantage over those individuals that do not flower every year.

The third trend which is apparent in the individual level phenologies is the occurrence of pulsing (i.e. gaps in the flowering season of an individual in which no flowers are at anthesis). Flowering pulses were observed in five of the six grassland species (all except *Raoulia monroi*) and three of the five riverbed species (pulses were not observed in *R. australis* or *R. haastii*). No flowering pulses were observed in alpine species. It is notable that, with the exception of *Gnaphalium traversii*, the pulses occur in species flowering later in the season. This pattern of distribution suggests that these pulses may occur due to lack of selection pressure at the end of the flowering season, which allows a

facultative response to favourable climatic conditions. The lack of pulses in alpine species is to be expected, as late flowering individuals are unlikely to set seed due to abortion or lack of pollinators late in the season (e.g. Webb, 1976; Kudo, 1993). The lack of pulsing in *R. haastii* and *R. australis* is consistent with the pollinator competition hypothesis of staggered flowering times in the riverbed species. *R. haastii* and *R. australis* both flower in the middle of the season, so that any late flowers are likely to be severely disadvantaged due to high interspecific pollen transfer. By contrast, late flowers occurring in *R. hookeri* and *H. depressum* are unlikely to be disadvantaged in the same manner, since they flower last in the season. Pulsing may therefore benefit individuals in these species since late flowers may receive higher visitation rates or avoid high levels of predation that may occur during the main flowering period. The potential cost to the plant is the occurrence of frosts which may result in the abortion of capitula. This appeared to be a frequent occurrence in some species (particularly *O. leptophyllus*) in which large numbers of aborted buds coincided with the occurrence of frosts in April-June (Figure 3.7). The occurrence of pulsing may therefore represent the balance between the cost incurred through bud abortion and the benefit gained through any seed which is produced.

The pattern of pulsing in *Gnaphalium audax* also suggests that physiology may be important in producing pulses in this species. The pulses in *G. audax* in the majority of individuals occurred during February in both years sampled. This pattern might occur if early flowering pulse was the result of buds initiated in the previous autumn, while the late flowering pulse resulted from buds initiated in the current season.

Cluster Patterns

The occurrence of secondary aggregations of capitula (referred to here as clusters) is widely spread across many tribes in Compositae (Claßen-Bockhoff, 1996). Burtt (1961) and Claßen-Bockhoff (1996) suggest that this aggregation of capitula may allow seed predators to be restricted to a small unit. This hypothesis appears to be supported by observations of *Leucogenes grandiceps* in which all instances of predation were observed in the central capitulum of the clusters. This selection pressure may be particularly prevalent where there is pressure to increase floral displays. By simply increasing the number of florets per head the whole capitulum may be susceptible to damage, while in a cluster, which would achieve the same display size, the insect would be restricted to one capitulum. Straw (1989) showed that more than one species of Tephritid may occur in

large heads, but typically only a single species would predate small heads. Thus by grouping capitula into a cluster, the predators are effectively restricted to one capitula, while still allowing a large floral display to be attained. This method would be particularly effective if the seed predators are univoltine, and if there is some delay between opening of the first and subsequent capitula, as was observed in *Leucogenes grandiceps*.

Selection for a larger floral display may also have been important in the evolution of the secondary aggregation in *Ozothamnus leptophyllus*. The cymose structure of the clusters in this species provide, in addition to a large floral display, some physical separation between the heads since each capitulum is presented on a small branch. Thus, even if some of the capitula are damaged by herbivores or other forms of mechanical damage, the physical separation of the capitula would mean that the majority of other capitula will be unaffected. This suggestion was first put forward by Burtt (1961) as a feature of this type of synflorescence.

In contrast to *Leucogenes grandiceps* and *Ozothamnus leptophyllus* in which secondary aggregation appears to be a packaging strategy to maximise floral display and/or minimise predator or mechanical damage, the cluster in *Gnaphalium audax* cannot be attributed to these selective pressures as no floral visitors (which include predators) were observed. An alternative hypothesis is that the secondary aggregation represents a packaging strategy to maximise the efficiency of resource utilisation. Both *G. traversii* and *G. audax* are opportunistic species (see below) which release seed from elevated positions. In *G. traversii* the flower bearing stem elongates after anthesis raising the solitary head up to 9 cm above the rosette (Drury, 1972). By comparison, the flowering stem of *G. audax* elongates prior to flowering, may be up to forty centimetres long and contains nine or more capitula in the terminal cluster and occasionally one or two additional clusters lower down the stem (Drury, 1972). If the cost necessary to produce the flowering stem is assumed to be equal in both species, and this cost is estimated on the basis of the length of the stem, the cost per capitula more than twice as great in *G. traversii* than in *G. audax*. In addition the more numerous, small capitula per cluster of *G. audax* effectively ensure that seed is released over a long period, an important adaptation for an opportunistic species. The production of a cluster may also represent a packing strategy to maximise the amount of seed produced by each axial meristem. The single capitulum per flowering stem in *G. traversii* produces on average 110 seed, while, on average, the combined capitula in

each cluster of *G. audax* will produce over 430 seed. Thus by producing a cluster of capitula, *G. audax* is potentially able to produce four times as many seed from each axial bud.

Capitula and Floret Patterns

A feature common to all Compositae heads is the crowded nature of the inflorescence. In outcrossing species this has important implications for the capitula's ability to achieve as both maternal and paternal functions, since any floral structures may potentially remove outcross pollen before it can be deposited on the stigma, or self pollen before it can be dispersed effectively (Lloyd and Webb, 1986; Webb and Lloyd, 1986). In the species examined, three features of the floret phenology appear to be adaptations that may reduce pollen wastage, geitonogamy, or other interference between neighbouring florets. First, the protandrous development of the tubular florets separates the male and female functions in each floret. This allows the pollen and stigma to be presented in the same position, and prevents the autogamous self-pollen from being wasted or clogging the stigma. Second, the curling of the style arms (e.g. *Ozothamnus leptophyllus*, *Helichrysum filicaule*), a feature traditionally associated with selfing (e.g. Müller, 1883), or a short style arm (e.g. *Raoulia mammillaris*, *R. grandiflora*), would also reduce interference between neighbouring florets. A long style arm which did not curl would overlap, and therefore interfer, with many florets in the small heads, while the curled or short styles arms are restricted to an area above their own corolla. The third feature, is the withdrawal of floral parts when their function is complete. The stamen tube, style, and in some species, the corolla tube, all withdraw so that they do not interfere with the next phase of the same floret or the function of the adjacent florets. Lloyd (1972) also suggested that the withdrawal of floral parts in *Cotula* correlated with outcrossing would reduce interference. He also suggested that the withdrawal of the floral parts may serve to reduce the potential for pollinators to be distracted while visiting the capitulum.

A final feature of the capitula and floret phenology that may reduce intra-capitula geitonogamy and interference is the presentation of the later opening florets at a progressively greater height, and in a few species (e.g. *Helichrysum intermedium*, *Raoulia glabra*) the bending of the outer florets. Both these features would reduce the amount of interference of early florets on later opening florets, by increasing the spatial separation between the florets.

A feature that was common to the species in which the corolla of the tubular floret was presented above the level of the pappus, was the curling of the corolla lobes. This may be important in allowing florets to be closely placed in the capitulum. The curling of the corolla lobes would also reduce the potential for the corolla lobes of neighbouring florets to overlap. Such overlap could potentially distract pollinators and/or discourage them from visiting subsequent florets in that capitulum.

The final feature of the capitula phenology that occurred in all study species, and is well known in other Compositae (e.g. Neff and Simpson, 1990), is the staggered opening of the tubular florets, with only a few tubular florets reaching anthesis at anyone time. In the study species each group of tubular florets usually began presenting pollen when the previous group entered the female phase. This characteristic of the Compositae is generally accepted as a method of maximising the time during which pollen is presented (e.g. Lloyd and Webb, 1986; Yeo, 1993). However, such a staggered opening would also mean that the styles of each floret reached anthesis at a different time, and would therefore be subject to a unique sequence of insect visiting, potentially allowing each group of florets to receive pollen from a different pollen donor. Thus the phenological patterns observed here support Burtt's (1961) suggestion that the Compositae head represents a highly efficient system to explore different genetic recombinations.

Adaptive Functions of the Patterns

In addition to the general patterns which were observed in all the study species, the patterns at the individual, cluster, capitulum, and floret levels combine with the breeding system to produce different strategies that achieve reproductive success in different circumstances.

(1) Opportunist Species

The most striking adaptive strategy is found in *Gnaphalium traversii* and *G. audax*. These species are opportunistic (Drury, 1972), establishing in small openings in grassland or on disturbed sites. As such, these species appear to have developed a number of features which differ from the other study species (Table 3.6). Perhaps the most important of these are adaptations for self fertilisation. These two species are self-compatible, while the other species in this study (in the Cass populations at least) are self-incompatible (pers. comm. R. McKenzie). This is supported by the pollen-ovule ratios, which have been shown to be

a conservative indicator of breeding systems (Cruden, 1977). The two *Gnaphalium* species had pollen-ovule ratios of only 35, a figure which places them in Cruden's (1977) obligate autogamy category, and which has been found in other selfing Compositae (Short, 1981). All other study species have pollen-ovule ratios in excess of 400, placing them in the outcrossing category (Cruden, 1977). The *Gnaphalium* species also differed in the relative proportions of filiform to tubular florets. While many study species had an approximate equal, or slightly tubular biased floret ratio, the *Gnaphalium* species had 13-15 filiform florets per tubular floret. In addition, they set seed in an average of 87% of florets, compared to a maximum of 47% (in *Helichrysum intermedium*) in the other study species. These differences in breeding systems are reflected in the distinct female biased gender estimates obtained for the two *Gnaphalium* species, compared to the other species. The difference in breeding systems also correlates with phenological differences at the capitulum and floret levels in both *Gnaphalium* species, and in *G. audax*, at the population/individual level. At the capitulum level, the most notable difference of *Gnaphalium* species when compared to other study species was the position of pollen presentation. In the other species the pollen was presented well above the level of the styles of the filiform florets and was released gradually. By comparison, in *G. traversii* and *G. audax*, the stamen tube did not extend much (if at all) above the level of styles of the filiform florets, and pollen presentation appeared to occur rapidly (hence the mass of pollen visible in Plate 19B).

The second phenological difference is the length of the flowering season, particularly that of *G. audax* which flowered for 16 to 22 weeks. This long flowering period must be, at least partially, the result of the numerous capitula in each of the clusters, which due to the staggered opening, ensures that each flowering stem may be active for forty days or more[1]. It is notable that under glasshouse conditions, *G. traversii* may also develop up to four capitula on each flowering stem (Drury, 1972). Combined, these phenological and breeding system characteristics ensure that *G. audax* and *G. traversii* are able to achieve a high level of set seed in the absence of floral visitors. This is supported by glasshouse observations, in which the majority of filiform styles withdrew soon after the first tubular florets opened, indicating that self-pollination had occurred. This phenological pattern was

[1] Calculated on the assumptions of (1) nine capitula per cluster, each opening when the previous capitulum reaches late anthesis. (2) six capitula opening as pairs, and (3) a seven to nine day duration for the anthesis for each group.

not observed in any other species. The long flowering season of *G. audax* also ensures a long period of seed release, since seed will be released as each successive capitulum as it reaches maturity. The ability to achieve self-pollination and release seed over a long period are important characteristics for an opportunistic species. Other characteristics of *G. audax* and *G. traversii* also meet Baker's (1965) requirement for an "ideal (?) weed", including vegetative growth combined with brittleness (by means of stolons), a rosette growth form, and a short life cycle. Thus the phenological features contribute to the

	Gnaphalium audax and *G. traversii*	Other Study species
1*	Vegetative reproduction through the production of daughter rosettes, combined with brittleness. Stoloniferous.	Often have vegetative spread with adventitious rooting, but are not brittle or common form of reproduction. Not stoloniferous.
2*	Rosette growth form.	Mat, shrub, or cushion growth forms.
3*	Short lived - two to three years (Drury, 1972).	Most long lived, > three years.
4*	Self-compatible. (pers. comm. R. McKenzie)	Self-incompatible. (pers. comm. R. McKenzie).
5	Low pollen-ovule ratio.	High pollen-ovule ratio.
6*	High proportion of florets set seed (87%).	Low proportion of florets set seed (2-47%).
7	High proportion of filiform florets per capitulum - filiform biased.	Proportion of florets even, or tubular biased.
8	Most filiform florets retract after one or two tubular florets have presented pollen.	Most filiform styles presented for a longer period than pollen presentation.
9	Corolla of tubular florets not showy. Only just level with top of pappus.	Corolla of tubular florets showy and forming a distinct cup or tube during anthesis, usually above level of involucral bracts and pappus.
10	Capitula lacking radiating involucral bracts, showy pappus hairs, and scent	Capitula with showy radiating involucral bracts and/or pappus and/or scented.
11	Pollen presented level with styles of the filiform florets, just above pappus.	Pollen presented well above top of pappus.
12	No floral visitors observed	Numerous floral visitors to most species.
13*	Flowering season in *G. audax* up to 22 weeks	Flowering season usually 6-12 weeks, up to 18 weeks
14	Seed released above level of vegetative growth.	Seed in most species released at same height as vegetative growth.

Table 3.6: The adaptive vegetative and reproductive features of *Gnaphalium audax* and *G. traversii* compared to the features of the other species included in this study. * corresponds to characteristics of an ideal weed (Baker, 1965; Baker, 1974).

adaptation of these species to an opportunistic life style. (They can not be termed weeds, since they appear to compete poorly in modified habitats.) It is interesting to note

characteristics probably associated with outcrossing are present in these species; both *Gnaphalium* species posses sensitive stamens, and nectar was observed in *G. audax*. The presence of these features indicates that these two species have probably evolved from an outcrossing ancestor.

(2) Reduced geitonogamy and interference

Burtt (1961) suggested that the Compositae head represented a highly efficient system to explore genetic recombination. This must be particularly true of a heterogamous capitulum, in which the protogynous development of the capitulum as a whole provides an opportunity for outcrossing to occur before pollen is presented. The placement of the filiform florets to the outside of the capitulum may also provide some degree of approach herkogamy, since an approaching insect may deposit pollen on the peripheral styles before it encounters the pollen being presented by later opening florets closer to the centre of the capitulum. This is particularly important in the tightly packed capitulum where the chances of geitonogamy are high. The effectiveness of this system is evident in the higher proportion of seed set by the filiform florets compared to the hermaphroditic tubular florets. A secondary benefit of the peripheral placement of the filiform florets may result from the tendency for seed predators to attack the centre of the capitulum (pers. obs.). Thus, by virtue of their peripheral placement, the outer florets may escape predation, and may continue to develop and release their seed.

The curling of style arms has traditionally been associated with self pollination both in species from other families (e.g. Klips and Snow, 1997), and within the Compositae (e.g. Müller, 1883; Yeo, 1993). However, the occurrence of this phenomenon in self-incompatible, outcrossing species, such as those in this study and *Veronia stenostegia* (Burtt, 1961), suggests that the curling of the style arms may perform other functions. It was suggested above that style arm curling may help to reduce interference between neighbouring florets in the capitulum. However, this could equally be achieved by reducing the length of the styles arms, as was observed in *Raoulia grandiflora* and *R. mammillaris*. Shortening of the style arms would also potentially reduce the cost of both producing the style, and maintaining the style during anthesis. Thus selfing or reduced interference fail to explain adequately the significance of this phenomenon. It is therefore suggested that the style curling may help to prevent interference in species with long style arms. In these species the long style arms ensure that a fresh stigmatic surface,

unclogged by self pollen, is available during most of anthesis. It is hypothesised that the gradual elongation and curling of the style arms will allow the gradual exposure of fresh stigmatic surfaces (i.e. stigmatic surfaces which has not been clogged by self pollen) while still preventing self interference.

Some evidence in support of this hypothesis is provided by the distribution of the style curling. The species which were observed to have the greatest rate of insect visitation (i.e. the riverbed species) had prominently curled filiform styles, while the alpine species, *Raoulia grandiflora* and *R. mammillaris*, had short style arms, and were less frequently visited. Thus, while geitonogamy undoubtedly occurs in the alpine species, the lower density of insects in the alpine environment (McCoy, 1990) means that this may not be as prevalent as on a riverbed, particularly compared to the *Raoulia* mats, where the capitula are very closely spaced. Additionally, the foraging behaviour of the insects on the riverbed would also increase the level of geitonogamy, as they were frequently observed to visit large numbers of capitula on one plant, and usually walked between capitula. The habit of walking, rather than flying, between capitula would potentially increase the level of geitonogamy, as many insects are known to remove pollen during flight (e.g. Holloway, 1976; Proctor *et al.*, 1996). It is notable that the alpine species *Leucogenes grandiceps* also has style arms of the longer curling type. The clustered capitula of this species could be expected to increase the level of geitonogamy experienced by this species. Thus it appears that the distribution of longer curling style arms fits the patterns that would be expected if they provided a selective advantage under conditions of high levels of geitonogamy.

The male function of the capitulum appears to be subject to a strong selection pressure due to the generalist nature of the pollinators, and their habit of visiting numerous capitula on a single plant, particularly in the riverbed species. In this habitat the high pollinator numbers and closely spaced capitula may be reducing the male fitness via pollen wastage. The species appear to have responded to this selective pressure in two ways: firstly by separating the male and female functions, thus allowing independent adjustment of the amount of pollen and ovules produced, or secondly, by changing the timing of pollen presentation. The tubular florets of *Raoulia tenuicaulis* and *R. hookeri* were found to set no seed, while less than 1% of tubular florets in *R. australis* set seed. Thus, in these three species, the structurally hermaphroditic tubular florets are functionally male. This shift in function is correlated with phenological changes, most notably the lack of or very minimal,

spread of the style arms, the lack of or very brief presentation of the style above the corolla, and the rapid collapse of the corolla following the male phase of anthesis. These changes would reduce pollen wastage in later opening tubular florets, and allow the independent adjustment/selection on the male and female functions.

In *Raoulia haastii* the small size of the capitulum may restrict the number of florets that can occur in any one capitulum. As a consequence the number of filiform and tubular florets may not be able to be adjusted independently. Thus the maintenance of the female function in the tubular florets would be important for the female fitness of an individual. However this means that the male function of the tubular florets may be reduced by the high levels of geitonogamy. It is therefore hypothesised that the small capitulum size has allowed the development of the capitulum phenology in which one or two tubular florets reach anthesis first. This would benefit the individuals by reducing the amount of pollen wastage within a head, and given the high level of synchrony between the adjacent flowers upon a mat (pers. obs.), between adjacent capitula on a plant. In addition, since the filiform florets do not usually open until late in the male phase or early in the female phase of the first tubular floret, this phenological pattern would still provide a period when the filiform florets are able to receive outcross pollen, without the interference from self pollen (at least from the same and neighbouring capitula). This pattern would also provide a similar period for the first tubular floret, potentially increasing the ability of this floret to fulfil its female function.

Thus the adjustment of phenological times in *R. haastii*, and the specialisation of the tubular florets in *R. tenuicaulis, R. australis,* and *R. hookeri* appear to have resulted from selection to reduce pollen wastage, while the long curling of the style arms appears to reduce stigma clogging.

(3) Pollination specialisation

(3.1): Homogamous capitula

The occurrence of homogamous capitula (i.e. containing only tubular florets) in *Ozothamnus leptophyllus* and *Helichrysum depressum* is probably the result of two independent events. Despite Allan's (1961) statement that filiform florets are present in *O. leptophyllus* none were observed in this study, or by Breitwieser and Ward (1997). The closely related Australian species, *O. ledifolius*, *O. ericifolius* and *O. alpinus,* also lack

filiform florets (pers.comm. J.M. Ward). It is therefore hypothesised that the absence of filiform florets in *O. leptophyllus* is the result of an ancestral condition present at the time this species established in New Zealand. In contrast some populations of *H. depressum* are known to contain individuals with heterogamous heads (pers.comm. J.M.Ward), as do most of the other New Zealand species of *Helichrysum*. Examination of preserved material of *H. depressum* with heterogamous capitula indicated that the styles of the filiform florets are presented well below the position of pollen and style presentation in the tubular florets. On the basis that butterflies were observed to be the main floral visitors, it is hypothesised that the more accurate foraging behaviour of the butterflies (frequent contact with the reproductive structures is only likely with the mouth parts and possibly the legs), and poor placement of the filiform styles, has resulted in the loss of filiform florets for two reasons. Firstly, butterflies are unlikely to probe the very narrow filiform florets if they do not contain nectar. While this cannot be confirmed for *H. depressum*, nectar was not observed in the filiform florets of the other study species. Secondly, the more precise method of operation by the butterfly means it would be unlikely to contact the filiform styles. Thus, there would be no selective advantage in maintaining filiform florets under these conditions. The morphology of the corolla in the tubular florets in *H. depressum* also suggests that it may be adapted to butterfly pollination. In this species the corolla forms a long narrow tube which gradually tapers, a morphology associated with butterfly pollination (Faegri and van der Pijl, 1979), whereas most of the other species had a cup-shaped corolla which tapered rapidly a short distance down the corolla, a morphology that would allow a variety of insects to access the nectar. It is interesting to note that *H. dimorphum*, the other New Zealand *Helichrysum* species with homogamous capitula, is strongly scented and visited by night-flying moths (Given, 1983). Given the morphological similarity between butterflies and moths, it could be expected that they would be equally precise, and thus may have also allowed the loss of filiform florets in this species. It is possible that the loss of filiform florets may have occurred in a common ancestor, as in two recent systematic studies *H. depressum* and *H. dimorphum* have been found to have a high level of similarity (Breitwieser, 1993; Breitwieser and Ward, 1993). However, a third recent study found *H. dimorphum* to be more similar to *H. filicaule* than to *H. depressum* (Haase *et al.*, 1993).

(3.2): Fly pollination

Another species which appears to show adaptations to a specific type of pollinator is *Raoulia mammillaris*. This species was observed to be visited almost exclusively by flies. The scent of this species was distinct from that of the other study species, but appears to match the description of the scent in the mainly fly pollinated species *Leontopodium alpinum* Cass. (Erhardt, 1993). Thus the scent and observations of floral visitors suggest that this species may be adapted to fly pollination. It is notable that the proportion of filiform florets setting seed (87%) is second only to the two *Gnaphalium* species, yet only 2% of the tubular florets set seed. This indicates that selection for functionally unisexual florets may be occurring. This would be an advantage in a species that is predominantly visited by pollen and nectar feeding insects (Holloway, 1976; Proctor *et al.*, 1996), since it would allow the separate adjustment of ovule and pollen production. The observation of a large number of small pollen grains may also represent an adaptation to pollen feeding insects, however this needs to be confirmed by the examination of pollen from other populations (there is a high incidence of hybridism at the site from which the material was collected).

A phylogenetic constraint

The release of pollen by the gradual elongation of the style, or following the triggering of the sensitive stamen by a pollinator, is well documented in the Compositae (e.g. Halsted, 1889; Small, 1915; 1917b; 1917c; Yeo, 1993). Lloyd (1979) suggested that these mechanisms provide an efficient system for presenting pollen which obviates the need for many polliniferous florets. Lloyd also noted that the only way the Compositae can increase the number of seed produced is to increase the number of florets. Thus the optimal number of ovuliferous florets may exceed the optimal number of polliniferous florets. This statement is supported by the *Gnaphalium* species, in which the large number of filiform florets probably resulted from the selection for a greater seed production. However, the higher proportion of tubular florets in the outcrossing species, the low level of seed set in these florets, and the occurrence of functionally male tubular florets, indicate that, contrary to Lloyd's suggestion, selection for an increase in the pollen-ovule ratio may result in more polliniferous florets. This discrepancy may be explained by the short life span of trinucleate pollen (Brewbaker, 1967; Faegri and van der Pijl, 1979). Hoekstra and Bruinsma (1975) state that in conditions of high temperatures or humidity Compositae pollen may only be viable for a few hours after anthesis. Thus, the Compositae may be

unable to produce more pollen per floret, since the pollen would potentially be inviable before it is dispersed, unless the rate of pollen release was increased. However, increasing the rate of pollen dispersal would be counter productive since this would potentially increase the level of geitonogamy, and probably would not increase the number of pollinator visits on which pollen could be dispersed. It is therefore hypothesised that the short pollen life span imposes a phylogenetic constraint on the Compositae, such that in order to increase the number of pollen grains produced and effectively dispersed, they must produce more polliniferous florets, rather than producing more pollen grains per floret, or presenting the pollen from a single floret over a longer period. This hypothesis is supported by the pattern of pollen presentation in this study, with each group of tubular florets being observed to remain in male phase no longer than two days. Other studies also report pollen presentation of one to two days (e.g. Jones, 1978; Garnock-Jones, 1986; Neff and Simpson, 1990; Cabrera and Dieringer, 1992), with only one reference reporting pollen presentation by a single floret lasting for up to three days (Berry and Calvo, 1989). This potential constraint on the evolutionary patterns of the Compositae capitulum appears to have been overlooked, however similar constraints have been hypothesised for flowers of the Umbelliferae, *Ranunculus* and *Gentiana* (Webb, 1984).

Conclusion

Considerable variation in flowering phenology of the species of the New Zealand Inuleae was observed despite the generalised pollinator fauna, and the close relationship between these taxa. The success of the Compositae must therefore also partially be attributed to the flexibility of the capitulum. This flexibility allows variation in the number and type of florets present, and as a consequence in the capitulum phenology and breeding system. The flexibility is enhanced by the presence of filiform florets which appear to allow the independent adjustment of the male and female functions in response to the selection pressures of geitonogamy or interference, particularly when the tubular florets are functionally male. The flexibility of the capitulum also appears to have allowed the evolution of adaptations to specialist pollinators or different life histories.

Taxonomically, this study highlights the importance of phenological and pollination studies because of the insight these studies give into the possible functional significance of breeding systems and features of the floral morphology such as style morphology and floret bending. The range of movement observed in the floret phenology also indicates the

importance of an understanding of floral phenology in order to obtain truly comparable measurements of floral features.

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Appendix 1: Collection Data

Anaphalis keriensis (A.Cunn.) C. Webb. New Zealand: Nelson, Anatoki Valley, CANU 37664, A.D.Wilton 235, 15/12/94.

Anaphalis rupestris C. Webb. New Zealand: Otago, Shag Pt., J.Ward 91135/2.

Anaphalis subrigida (Colenso) C. Webb. New Zealand: Central North Is., Taihape, J.Ward 94218.

Anaphalis trinervis (G.Forst.) F. Muell. New Zealand: Wellington, Rimataka Ranges, J.Ward 94245.

Cassinia aculeata R. Br. Australia: Tasmania, Mt. Hartz National Park, J.Ward 94115; Australia: N.E. Tasmania, near St Mary, J.Ward 96103.

Cassinia longifolia R. Br. Australia: Tasmania, Pipers Brook, J.Ward 94101; Australia: N.E. Tasmania, near St Mary, J.Ward 96102.

Ewartia catipes (DC.) Beauverd. Australia: Tasmania, Central Plateau, near Lake Augusta, J.Ward 94081/1; Australia: Tasmania, Ben Lomond, J.Ward 94098/7.

Ewartia meredithiae (F. Muell.) Beauverd. Australia: Tasmania, Mt Field National Park, Tarn Shelf, J.Ward 94040/1; Australia: Tasmania, Mt Hartz National Park, J.Ward 94118/1.

Ewartia planchonii (Hook.f.) Beauverd. Australia: Tasmania, Mt Field National Park, boardwalk to Rodway Hut, J.Ward 94043/5; Australia: Tasmania, Hobart, Mt Wellington, J.Ward 96100.

Ewartia sinclairii (Hook.f.) Cheeseman. New Zealand: Marlborough, Hodder, CANU 37722, A.D. Wilton 294, 18/1/95; New Zealand: Marlborough, Yeo Stream, CANU 37807.

Gnaphalium audax D.G.Drury. New Zealand: Canterbury, Banks Peninsula, CANU 37642, A.D. Wilton 181, 22/3/94; New Zealand: Canterbury, Cass, CANU 37550, A.D. Wilton 18, 15/10/93.

Gnaphalium involucratum G.Forst. New Zealand: Westland, Lake Brunner, CANU 37594, A.D. Wilton 114, 3/5/94; New Zealand: Otago, Waitaki, J.Ward 91139.

Gnaphalium mackayi (Buchanan) Cockayne. New Zealand: Nelson, Cobb Valley, CANU 37674, A.D. Wilton 245, 13/12/94; New Zealand: Otago, Remarkables, CANU 37747, A.D. Wilton 307, 28/1/95.

Gnaphalium nitidulum Hook. f. New Zealand: Marlborough, Island Saddle, CANU 37801, A.D. Wilton 143, 3/9/94; Australia: N.S.W., Kosciusko National Park, J.Ward 96045/2.

Gnaphalium traversii Hook. f. New Zealand: Canterbury, Cass, CANU 37534, A.D. Wilton 0, 14/10/93; New Zealand: Marlborough, Hodder, CANU 37715, A.D. Wilton 286, 18/1/95.

Haastia pulvinaris Hook. f. New Zealand: Marlborough, Balaclava, CANU 37598, A.D. Wilton 121, 3/9/94; New Zealand: Canterbury, Mt Edison, CANU 37774, A.D. Wilton 365, 3/11/94.

Haastia sinclairii Hook. f. New Zealand: Canterbury, Mt Potts, CANU 37682, A.D. Wilton 253, 21/12/94; New Zealand: Canterbury, Broken River, CANU 37796, A.D. Wilton 116, 3/9/94.

Helichrysum bellidioides (G.Forst.) Willd. New Zealand: Canterbury, Broken River, CANU 37575, A.D. Wilton 95, 2/9/94; New Zealand: Canterbury, Banks Peninsula, CANU 37640, A.D. Wilton 179, 22/3/94.

Helichrysum coralloides (Hook. f.) Benth. et Hook. f. New Zealand: Marlborough, Hodder, CANU 37717, A.D. Wilton 288, 18/1/95; New Zealand: Canterbury, Mt Lyford, 37812, A.D. Wilton 331, 1/9/95.

Helichrysum depressum (Hook. f.) Benth. et Hook. f. New Zealand: Canterbury, Cass, CANU 37535, A.D. Wilton 3, 14/10/93; New Zealand: Canterbury, Serpentine Cr, CANU 37618, A.D. Wilton 156, 3/9/94.

Helichrysum dimorphum Cockayne. New Zealand: Canterbury, Poulter River, CANU 37706, A.D. Wilton 177, 15/1/94.

Helichrysum filicaule Hook. f. New Zealand: Canterbury, Cass, CANU 37579, A.D. Wilton 99, 2/9/94; New Zealand: Canterbury, Banks Peninsula, CANU 37638, A.D. Wilton 177, 22/3/94.

Helichrysum intermedium G.Simpson. New Zealand: Canterbury, Dry Stream, CANU 37573, A.D. Wilton 93, 2/9/94; New Zealand: Marlborough, Balaclava, CANU 37611, A.D. Wilton 136, 3/9/94.

Helichrysum lanceolatum (Buchanan) Kirk. New Zealand: Canterbury, Banks Peninsula, CANU 37635, A.D. Wilton 174, 22/3/94; New Zealand: Marlborough, Isolated Hill, CANU 37728, H.Cochrane, 4/4/94.

Helichrysum parvifolium Yeo. New Zealand: Marlborough, Rag and Famish Stream, CANU 37599, A.D. Wilton 122, 3/9/94; New Zealand: Canterbury, Mt Lyford, CANU 37763, A.D. Wilton 327, 1/9/95.

Leucogenes grandiceps (Hook. f.) Beauverd. New Zealand: Canterbury, Broken River, CANU 37557, A.D. Wilton 39, 28/10/93; New Zealand: Fiordland, Scotts Basin, CANU 37751, A.D. Wilton 314, 26/1/95.

Leucogenes leontopodium (Hook. f.) Beauverd. New Zealand: , Mt Holdsworth, J.Ward 94225.

Ozothamnus leptophyllus (G.Forst.) Breitw. et J.M.Ward. New Zealand: Canterbury, Cass, CANU 37546, A.D. Wilton 14, 15/10/93; New Zealand: Marlborough, Clarence

River, CANU 37615, A.D. Wilton 140, 3/9/94; New Zealand: Marlborough, Island Saddle, CANU 37788, A.D. Wilton 150, 3/9/94.

Ozothamnus obcordatus DC. Australia: Tasmania, East Coast, Bicheno, J.Ward 94113; Australia: Tasmania, Hobart, Grass Tree Hill, J.Ward 96125.

Ozothamnus rodwayi Orchard. Australia: Tasmania, Mt Hartz National Park, J.Ward 94117; Australia: Tasmania, Mt Field National Park, J.Ward 94035.

Pseudognaphalium luteoalbum (L.) Hilliard et B.L.Burtt. New Zealand: Canterbury, Porters Pass, CANU 37560, A.D. Wilton 49, 11/10/93; New Zealand: Marlborough, Balaclava, CANU 37793, A.D. Wilton 134, 3/9/94.

Pterygopappus lawrencei Hook. f. Australia: Tasmania, Central Plateau, Pine Lake, J.Ward 94077; Australia: Tasmania, Ben Lomond, J.Ward 94094.

Rachelia glaria J.M.Ward et Breitw. New Zealand: Marlborough, Barefell, CANU 35555.

Raoulia australis Hook. f. New Zealand: Canterbury, Cass, CANU 37538, A.D. Wilton 6, 14/10/93; New Zealand: Marlborough, Hodder, CANU 37718, A.D. Wilton 289, 18/1/95.

Raoulia bryoides Hook. f. New Zealand: Marlborough, Balaclava, CANU 37616, A.D. Wilton 148, 3/9/94; New Zealand: Canterbury, Mt Edison, CANU 37773, A.D. Wilton 364, 28/11/94.

Raoulia cinerea Petrie. New Zealand: Marlborough, Mt Barefell, JW 89091/1; New Zealand: Marlborough, Balaclava, CANU 37799, A.D. Wilton 132, 3/9/94.

Raoulia eximia Hook. f. New Zealand: Canterbury, Mt Plenty Ridge, CANU 37582, A.D. Wilton 102, 13/2/94; New Zealand: Canterbury, Poulter Range, CANU 37703, A.D. Wilton 274, 1/12/95.

Raoulia glabra Hook. f. New Zealand: Canterbury, Broken River, CANU 37576, A.D. Wilton 96, 2/9/94; New Zealand: Canterbury, Banks Peninsula, CANU 37648, A.D. Wilton 187, 22/3/94.

Raoulia grandiflora Hook. f. New Zealand: Canterbury, Middlebasin, CANU 37593, A.D. Wilton 113, 21/2/94; New Zealand: Marlborough, Island Saddle, CANU 37625, A.D. Wilton 163, 3/9/94.

Raoulia haastii Hook. f. New Zealand: Canterbury, Cass, CANU 37537, A.D. Wilton 5, 14/10/93; New Zealand: Canterbury, Tekapo, CANU 37650, A.D. Wilton 207, 4/4/94.

Raoulia hectorii Hook. f. New Zealand: Canterbury, Mt Dobson, CANU 37563, A.D. Wilton 57, 12/11/93; New Zealand: Canterbury, Ohau, CANU 37696, A.D. Wilton 267, 1/1/95.

Raoulia hookeri Allan. New Zealand: Canterbury, Cass River, CANU 37795, A.D. Wilton 117, 2/9/94; New Zealand: Marlborough, Wairau River, CANU 37630, A.D. Wilton 168, 3/9/94.

Raoulia mammillaris Hook. f. New Zealand: Canterbury, Mt Plenty Ridge, CANU 37588, A.D. Wilton 108, 13/2/94; New Zealand: Canterbury, Poulter Hill, CANU 37701, A.D. Wilton 272, 14/1/95.

Raoulia monroi Hook. f. New Zealand: Canterbury, Cass, CANU 37542, A.D. Wilton 10, 14/10/93; New Zealand: Canterbury, Banks Peninsula, CANU 37643, A.D. Wilton 182, 20/3/94.

Raoulia petriensis Kirk. New Zealand: Marlborough, Mt St Bathans, J.Ward 91150/1.

Raoulia subsericea Hook. f. New Zealand: Canterbury, Cass, CANU 37553, A.D. Wilton 21, 16/10/93; New Zealand: Marlborough, Balaclava, CANU 37608, A.D. Wilton 131, 3/9/94.

Raoulia subulata Hook. f. New Zealand: Canterbury, Broken River, CANU 37769, A.D. Wilton 334, 3/4/95; New Zealand: Canterbury, Princess Bath, CANU 37805, A.D. Wilton 340, 14/1/96.

Raoulia tenuicaulis Hook. f. New Zealand: Canterbury, Broken River, CANU 37577, A.D. Wilton 97, 2/9/94; New Zealand: Marlborough, Wairau River, CANU 37619, A.D. Wilton 157, 3/9/94.

Raoulia youngii (Hook. f.) Beauverd. New Zealand: Otago, Awakino, CANU 37699, A.D. Wilton 270, 2/1/95; New Zealand: Canterbury, Mt Potts, CANU 37687, A.D. Wilton 258, 21/12/94.

Raoulia "L". New Zealand: Otago, Cardrona, J.Ward 94026; New Zealand: Otago, Remarkables, CANU 37740, A.D. Wilton 303, 28/1/95.

Raoulia "M". New Zealand: Marlborough, Hodder, CANU 37714, A.D. Wilton 285, 18/1/95; New Zealand: Canterbury, Mt St Patrick, CANU 37595, A.D. Wilton 336, 27/11/94.

APPENDIX 2: STEM CHARACTERS

The characters and character states used in cladistic and numerical analyses are listed below. Characters which were excluded from the analyses, because of inconsistency or uncertain in their distribution, are marked by the abbreviation "EX". The abbreviations MPS and MSS are used for mature primary and mature secondary stem sections respectively. Consistency (CI) and Rescaled consistency index (RC) are given beneath each character for the consensus trees produced from Run 6.

(1) Pith thickening in tip sections. **(EX)**
1: none
2: collenchymatous
3: collenchymatous and lignified
4: lignified
CI = 0.143 RC = 0.036.

(2) Pith thickening in MPS sections. **(EX)**
As for character (1).
CI = 0.167 RC = 0.048.

(3) Pith thickening in MSS sections.
As for character (1).
CI = 0.6 RC = 0.

(4) Pith end wall appearance in tip sections. **(EX)**
1: grainy
2: fibrous
3: smooth pitted
CI = 0.133 RC = 0.

(5) Pith end wall appearance in MSS sections.
As for character (4).
CI = 0.667 RC = 0.

(6) Intercellular spaces in pith of MPS sections. **(EX)**
0: unfilled
1: partially filled
2: completely filled
CI = 0.333 RC = 0.

(7) Intercellular spaces in pith of MPS sections. **(EX)**
0: unfilled
1: filled or partially filled with dark middle lamella
2: filled or partially filled with pale middle lamella
CI = 0.250 RC = 0.083.

(8) Intercellular spaces in pith of MSS sections.
As for character (6).
CI = 0.200 RC = 0.067.

(9) Intercellular spaces in pith of MSS sections.
As for character (7).
CI = 0.143 RC = 0.036.

(10) Primary xylem.
0: some cells unlignified
1: all cells lignified
CI = 0.111 RC = 0.048.

(11) Vessel grouping - tangential aggregations of vessels in MSS sections.
0: absent
1: present
CI = 0.167 RC = 0.111.

(12) Vessel grouping - radial aggregations of vessels in MSS sections.
As for character (11).
CI = 0.333 RC = 0.167.

(13) Vessel grouping - clumped aggregations of vessels in MSS sections.
As for character (11).
CI = 0.5 RC = 0.

(14) Ray type in MSS sections. **(EX)**
1: none visible or uncertain
2: uniseriate
3: multiseriate
4: medullary
CI = 0.133 RC = 0.051.

(15) Axial Parenchyma in secondary xylem of MSS sections. **(EX)**
0: not observed
1: observed in one specimen
2: observed in both specimens
CI = 0.077 RC = 0.003.

(16) Growth rings in MSS sections.
0: absent
1: present
CI = 0.143 RC = 0.095.

(17) If present, type of growth ring.
1: type 1
2: type 2
3: type 3
4: type 4
5: type 5
CI = 0.5 RC = 0.4.

(18) Anomalous cambium activity apparent in MSS sections.
0: absent
1: present
CI = 1 RC = 1.

(19) Maximum vessel diameter (μm) in MSS sections. (EX)
1: 5 to 15
2: 15 plus to 25
3: 25 plus to 35
4: 35 plus to 45
5: 45 plus
CI = 0.176 RC = 0.031.

(20) Fibres in phloem of MSS sections.
0: absent
1: present
CI = 0.111 RC = 0.100.

(21) If present, type of fibre in MSS sections.
1: mass - large lumen
2: separate - large or small lumen
CI = 0.143 RC = 0.167.

(22) Casparian strip present in endoderm of tip and/or MPS sections.
0: absent
1: present
CI = 0.167 RC = 0.083.

(23) Endoderm walls in tip sections.
1: unthickened
2: radial walls
3: radial and outer tangential
4: all
CI = 0.667 RC = 0.

(24) Endoderm walls in MPS sections (EX)
As for character (23).
CI = 0.167 RC = 0.064.

(25) Endoderm walls in MSS sections.
As for character (23).
CI = 0.2 RC = 0.08.

(26) Endoderm lignified
0: unlignified
1: lignified
CI = 0.167 RC = 0.097.

(27) Cortex type in MPS sections
1: cells approximately homogeneous
2: clearly demarcated layer of large outer cells
3: clearly demarcated layer of large inner cells
CI = 0.250 RC = 0.083.

(28) Cortex spacing in MPS sections (other than small spacing at cell corners).
1: Prominent spaces between the outer cortex cells
2: Large spaces directly beneath the epidermis.
3: Large spaces in the outer cortex: one or two layers of cells inside epidermis.
4: Prominent spaces throughout the cortex, at the cell corners.
5: Small spaces between outer cortex and epidermis
CI = 0.312 RC = 0.163.

(29) Aerenchymatous spaces in cortex of MSS sections.
0: absent
1: present
CI = 1 RC = 0.

(30) Lignified cells present in the cortex of MPS sections.
0: absent
1: present
CI = 0.143 RC = 0.071.

(31) Resin canals present in cortex and leaf sheath.
0: absent
1: present
CI = 1 RC = 1.

(32) Epidermal cell shape in tip sections - flat. **(EX)**
0: not observed
1: observed in 1 specimen
2: observed in both specimens
CI = 0.095 RC = 0.017.

(33) Epidermal cell shape in tip sections - tall. **(EX)**
As for character (32).
CI = 0.077 RC = 0.009.

(34) Epidermis cell shape in tip sections - square. **(EX)**
As for character (32).
CI = 0.083 RC = 0.

(35) Epidermis cell shape in tip sections - squashed. **(EX)**
As for character (32).
CI = 1 RC = 0/0.

(36) Epidermal cell shape in MPS sections - flat. **(EX)**
As for character (32).
CI = 0.077 RC = 0.017.

(37) Epidermal cell shape in MPS sections - tall. **(EX)**
As for character (32).
CI = 0.105 RC = 0.011.

(38) Epidermal cell shape in MPS sections square. **(EX)**
As for character (32).
CI = 0.091 RC = 0.024.

(39) Epidermal cell shape in MPS sections - squashed. **(EX)**
As for character (32).
CI = 0.182 RC = 0.018.

(40) Biseriate Hairs in tip sections. **(EX)**
1: absent
2: Swollen terminal cells
3: Terminal cells not swollen
4: 2 and 3
CI = 0.400 RC = 0.

(41) Biseriate hairs in MPS sections **(EX)**
As for character (40).
CI = 0.250 RC = 0.083.

(42) Stoma in epidermis of stem in Tip and/or MPS sections.
0: absent
1: present
CI = 0.167 RC = 0.111.

(43) If present, stoma type.
1: flat
2: raised
CI = 1 RC = 1.

(44) Cuticle striations in Tip and/or MPS sections. **(EX)**
0: absent
1: present
CI = 0.1 RC = 0.

(45) Cuticle ridges of type A in Tip and/or MPS sections. **(EX)**
0: absent
1: present
CI = 0.77 RC = 0.023.

(46) Cuticle ridges of type B in Tip and/or MPS sections. **(EX)**
0: absent
1: present
CI = 0.2 RC = 0.

(47) Cuticle ridges of type C in Tip and/or MPS sections. **(EX)**
0: absent
1: present
CI = 0.5 RC = 0.250.

(48) Outer layer in MSS sections - epidermis.
0: absent
1: present
CI = 0.333 RC = 0.294.

(49) Outer layer in MSS sections - endodermis
0: absent
1: present
CI = 0.250 RC = 0.

(50) Outer layer in MSS sections - cortex
0: absent
1: present
CI = 0.5 RC = 0.

(51) Outer layer in MSS sections - periderm
0: absent
1: present
CI = 0.2 RC = 0.162.

(52) If present, the position of the periderm in MSS sections.
1: outer phloem
2: outer cortex
CI = 0.167 RC = 0.

(53) Number of leaf traces and gaps in Tip and MPS sections. **(EX)**
Count.
CI = 0.167 RC = 0.048.

(54) Nodal state.
1: unilacunar
2: trilacunar
3: multilacunar
CI = 0.167 RC = 0.048.

(55) Arrangement of traces in cortex. **(EX)**
1: laterals ahead
2: traces even
CI = 0.250 RC = 0.1.

(56) The number of veins observed in the leaf sheath. **(EX)**
Count.
CI = 0.154 RC = 0.0204.

(57) Veins in the leaf sheath.
0: equal to number of traces
1: greater than number of traces
CI = 0.5 RC = 0.375.

(58) If the number of veins in leaf sheath greater than leaf gaps, the origin of the veins.
1: vascular cylinder
2: main vein
3: lateral vein
4: lateral and main
CI = 0.375 RC = 0.107.

(59) Sheath sclereids
0: none
1: scattered mesophyll
2: entire mesophyll
3: adaxial mesophyll
4: adaxial epidermis
5: 3 and 4
CI = 0.333 RC = 0.128.

(60) Number of bundle sheath layers.
Count.
CI = 1 RC = 1.

(61) Lignified cells in the bundle sheath.
0: none present
1: present - not to one particular area restricted
2: present - restricted to adaxial part of bundle sheath
CI = 0.400 RC = 0.

(62) Sclerenchyma caps in leaf sheath veins.
0: none
1: abaxial
2: adaxial
3: cylinder
CI = 0.333 RC = 0.

(63) Spherical crystals **(EX)**
1: Observed in one sample
2: Observed in two samples
CI = 0.111 RC = 0.012.

(64) Rod crystals **(EX)**
As for character (63).
CI = 0.400 RC = 0.

(65) Rhomboidal crystals **(EX)**
1: Observed in one sample
2: Observed in two samples
CI = 0.222 RC = 0.028.

(66) Irregular crystals **(EX)**
As for character (63).
CI = 0.250 RC = 0.062.

(67) Starch grains (EX)
As for character (63).
CI = 0.2 RC = 0.

(68) Silica bodies (EX)
As for character (63).
CI = 0.5 RC = 0.

Characters which were re-coded into binary stepped characters for the phenetic analysis were: 1, 3, 5, 9, 17, 21, 23, 25, 27, 28, 43, 57, 59, and 62.

Appendix 3: Matrix of Stem Characters

Character No.[A]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Anarup	2	1 | 2	4	1	3	2	1	1	1	0	1	1	0	4	0	1	1
Anasub	2	2	4	2	3	1 | 2	1	2	2	0	0	0	0	4	1	1	1
Anaker	1	1	4	1	3	2	1	2	1	0	0	0	0	4	1	1	1
Anatri	2	2	3	2	3	1	1	1	1	0	1	0	0	4	0	0	NA
Casacu	1 | 2	1	4	1	3	0 | 2	1	1 | 2	2	0	0 | 1	1	1	1 | 2 | 3	2	0	NA
Caslon	1 | 2	1 | 2	4	1 | 2	3	1	1	1 | 2	2	0	1	1	0 | 1	2 | 3	1	0	NA
Ewacat	1 | 2	1 | 3	4	1	3	1 | 2	1	1 | 2	2	0	1	0	0	1 | 4	1	0	NA
Ewamer	1	4	4	1	3	1 | 2	2	1 | 2	1 | 2	0	0	0	0	1 | 4	1	0	NA
Ewapla	1	2 | 3	4	1	3	1 | 2	1	1 | 2	1	0	0	0	0	1	1	0	NA
Ewasin	1	4	4	1 | 2	3	0 | 2	0 | 1 | 2	2	1 | 2	0	0 | 1	0	0 | 1	4	0	1	1
Gnaaud	2	3 | 4	4	3	3	0	0	1	2	0	0	0	0	1	0	0	NA
Gnainv	1 | 3	1 | 3	1	1	?	0	0	2	1	0	0	0 | 1	0	4	0	0	NA
Gnamac	1 | 2	1 | 2 | 3	1 | 3 | 4	1	1	1 | 2	1 | 2	1 | 2	2	0	0	0 | 1	0	1 | 4	0	0	NA
Gnanit	1	1	4	1 | 2	1	2	1	2	1 | 2	0	0	0	0	1	2	0	NA
Gnatra	2 | 3	3 | 4	?	2 | 3	?	0	0	?	?	0	0	0	0	1	0	0	NA
Haapul	4	4	4	3	3	2	2	2	2	0	0 | 1	0	0 | 1	1 | 2 | 3	1	1	1 | 4
Haasin	1	1 | 3	3 | 4	2	3	2	1 | 2	2	2	1	0 | 1	0 | 1	0	2 | 4	1	0	NA
Helbel	1 | 2	3	3 | 4	1	3	1 | 2	1	1 | 2	1 | 2	1	0	0 | 1	0	1 | 4	1	0	NA
Helcor	2	4	4	1 | 3	3	2	2	2	1 | 2	0	1	0	0	3	0	1	4
Heldep	1 | 2	4	4	3	3	1 | 2	1 | 2	1	1 | 2	0	0 | 1	0	0	1 | 3	2	1	5
Heldim	1	3	3	1	3	1 | 2	1 | 2	2	2	0	1	0	0	1 | 2	0	1	4
Helfil	1 | 3	4	4	1	3	1 | 2	2	1	1	1	0	0	0	1	0	0	NA
Helint	1	4	4	1 | 2	3	2	2	2	2	0	1	0	0	3	1	1	3
Hellan	2 | 3	4	4	1 | 2	3	1 | 2	1 | 2	1 | 2	1 | 2	0	0	1	0	3	2	1	1
Helpar	1 | 2	1 | 2 | 3	4	1	3	1 | 2	1 | 2	2	2	0	1	0	0	3	1	1	3
Leugra	2	2 | 4	4	1 | 2	3	2	1	1 | 2	1 | 2	0	0 | 1	0 | 1	0	2 | 4	0	0 | 1	1
Leuleo	2	4	4	1	3	0	0	2	2	1	1	0	0	3	0	0	NA
Ozolep	2	2	2	2 | 3	2	0 | 1 | 2	0 | 1	2	1	0	1	0 | 1	1	3	2	1	1 | 2 | 3
Ozoobc	1	1	4	1	3	1 | 2	1	1 | 2	2	0	0	1	0	1 | 3	1	0 | 1	1
Ozorod	2	1 | 2	4	2	3	2	1	2	1 | 2	0	1	0	0 | 1	3	2	1	4
Pselut	1 | 3	1 | 3	3	3	3	0 | 2	0 | 1	2	1	0	0	0	0	4	1	0	NA
Ptelaw	1 | 2	4	4	1	3	2	1 | 2	2	2	1	0	0	0	1	0	0	NA
Racgla	2	2	4	3	3	2	1	2	1	0	?	?	?	1	0	?	1
Raoaus	1	4	4	3	3	1 | 2	1 | 2	1 | 2	1 | 2	1	1	0 | 1	0	2 | 4	0	1	1 | 5
Raobry	1 | 2	4	4	1 | 3	3	1 | 2	2	1 | 2	2	1	0	0	0	1 | 3	0	0	NA
Raocin	2	2	3 | 4	1 | 3	3	1 | 2	1	2	1 | 2	0	0	0	0	1 | 4	2	0	NA
Raoexi	2	4	4	2 | 3	3	2	1	2	1 | 2	0	0 | 1	0	0	3	1	1	4
Raogla	1 | 2	2 | 3	3 | 4	1	3	2	1 | 2	1 | 2	1 | 2	0	0 | 1	0	0	4	1	0 | 1	1
Raogra	1	1 | 2	3 | 4	1	3	1 | 2	1	2	1 | 2	0	0	0	0	1	1	0	NA
Raohaa	1	4	4	1 | 2	3	0 | 1 | 2	0 | 2	0 | 1	0 | 2	1	1	0	0	1 | 2 | 3	1	1	2 | 3
Raohec	1	4	4	1 | 2	3	1 | 2	1	1	1 | 2	0 | 1	1	0	0	1 | 3 | 4	1	0	NA
Raohoo	2	2 | 4	4	1 | 2	3	2	1	1 | 2	1 | 2	0	0	0 | 1	0	1	2	1	1
Raomam	2	2 | 4	4	1 | 2	3	2	1 | 2	2	2	1	0	0	0	1 | 2	1	0	NA
Raomon	1 | 2	4	4	1 | 3	3	2	1 | 2	1 | 2	2	1	0	0	0	1	0	0	NA
Raopet	1	2	4	3	3	1 | 2	1	2	2	1	0	0	0	1	1	0	NA
RaospL	1 | 2	4	4	1	3	1 | 2	1 | 2	2	1 | 2	0	0	0	0	1	2	0	NA
RaospM	1 | 2	4	4	2 | 3	3	1 | 2	1	1 | 2	2	0	0 | 1	0 | 1	0	1	2	0	NA
Raosub	2	2 | 4	4	1 | 3	3	1 | 2	1 | 2	1 | 2	1 | 2	1	1	0	0	4	1	1	1
Raosuu	1	4	4	1	3	0 | 1 | 2	0 | 1 | 2	2	1 | 2	1	1	0 | 1	0	4	1	0	NA
Raoten	1 | 2	3 | 4	4	1	3	1 | 2	1	1 | 2	2	1	0 | 1	0	0	4	1	1	2
Raoyou	1	4	4	1 | 3	3	1 | 2	1 | 2	0 | 2	0 | 2	1	0	0	0	1 | 3	1	0	NA

[A]: see Appendix 2 for character names. Symbols indicate: or (|), and (&), unknown (?), not applicable (NA).

Character No.[A]	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Anarup	0	5	1	2	1	1	1	3	0	1	5	0	0	0	1	1	1
Anasub	0	3	1	2	1	1	1	1	0	1	5	0	0	0	1	1	1
Anaker	0	2	1	2	1	1	4	2	0	1	5	0	0	0	1	0	1
Anatri	0	3	1	2	0	1	1	1	0	1	0	0	0	0	1	0	1
Casacu	0	3	1	2	0	1	1	4	1	1 | 3	1 | 4	0	0	0	2	2	2
Caslon	0	4 | 5	1	2	0	1	1	4	1	3	1	0	0	0	2	1	2
Ewacat	0	1 | 2	0 | 1	2	0 | 1	1	1	2 | 4	0	1	5	0	0	0	0	2	1
Ewamer	0	1 | 2	0	NA	0	1	2 | 4	3	0 | 1	1	4	1	0	0	2	0	2
Ewapla	0	1 | 2 | 3	0	NA	0	1	1 | 4	3 | 4	0	1	0 | 4	0	0	0	2	0	1
Ewasin	0	2	1	2	0	1	3	3	1	3	0 | 1	0	1	0	2	1	2
Gnaaud	0	2	1	2	0	2	2	2	0	3	1 | 4	0	0	0	2	0	2
Gnainv	0	2 | 3	1	2	0	1 | 3	2 | 4	2	0	1	4	0	0	0	1	0	1
Gnamac	0	1 | 2	0	NA	1	1	1 | 4	4	0	1	5	0	0	0	1	1	2
Gnanit	0	1	0	NA	0	1	1	2	0	1	5	0	0	0	1	1	1
Gnatra	0	1 | 2	1	2	1	1 | 2	3 | 4	NA	0	1	4	0	0	0	2	0	1
Haapul	0	3	1	2	1	1	1	1	0	1	3	0	0	1	2	0	2
Haasin	0	2 | 3	1	2	1	1	1	1	0	1	3 | 5	0	0	1	2	0	1
Helbel	0	3 | 4	1	2	0	1	2 | 4	3 | 4	0	1	2 | 5	0	1	0	1	2	2
Helcor	0	2	1	2	1	1	1 & 4	NA	0 | 1	1	0 | 2	0	0	0	2	2	2
Heldep	0	2	1	2	0	1	1 | 4	NA	0 | 1	1	0 | 2	0	0	0	1	0	2
Heldim	0	3	0	NA	1	1	2	1	0	2	0	0	0	0	1	1	1
Helfil	0	2	1	2	0	1 | 3	3	3	1	3	1	0	1	0	1	2	2
Helint	0	2	1	2	0 | 1	1	1 | 4	NA	1	1	2 | 5	0	0	0	1	0	2
Hellan	0	3	1	2	0	3	3	3	1	3	0 | 1	0	1	0	1	1	1
Helpar	0	2	1	2	1	1 | 4	1 | 4	4	0	2	0 | 4	0	0	0	2	0	1
Leugra	0	1 | 2 | 3	1	1 | 2	0	1	1 | 4	3	0 | 1	1	0 | 2	0	0 | 1	0	2	1	2
Leuleo	0	2	1	2	0	1	3	4	0	1	0	0	0	0	1	1	1
Ozolep	0	2 | 3	1	2	0	1	4	4	1	1 | 3	3	0	0	0	2	0	1
Ozoobc	0	1 | 2 | 3	1	2	0	1	1	4	1	3	1	0	0	0	2	0	1
Ozorod	0	3	1	2	0	1	1	4	1	1	4	0	0	0	2	0	1
Pselut	0	2	1	2	0	1 | 2	1 | 2	2	0	3	1	0	0	0	2	0	1
Ptelaw	0	1 | 2	0	NA	0	1	2 | 4	4	0	1	0	0	0	0	2	0	1
Racgla	0	1	1	2	0	1	1	3	0	1	5	0	0	0	0	1	1
Raoaus	0	3	1	1	0	3	3	3 | 4	1	2	0	0	1	0	2	1	2
Raobry	1	1 | 2 | 3	1	1	0	1	3	NA	0	1	5	0	1	0	0	2	1
Raocin	0	1 | 2	0 | 1	2	0	1	1	1 | 2	0	1	2 | 5	0	0	0	2	0	2
Raoexi	0 | 1	3 | 4	1	1	0	1 | 3	4	4	0	1	0	0	0 | 1	0	0	1	1
Raogla	0	2 | 3	1	1	0	1	3	3 | 4	1	1	1 | 5	0	1	0	2	0	2
Raogra	0	1 | 2 | 3	0	NA	0	1	1	2	0	1	0 | 2	0	0	0	0	1	2
Raohaa	0	4 | 5	1	1	0	1	2 | 3	NA	0	2 | 3	0	0	1	0	2	1	2
Raohec	0	2	0	NA	0	1	1 | 2	4	0	1	0	0	0	0	1	2	1
Raohoo	0	3	1	1	0	1	3	3	1	1	0	0	0 | 1	0	1	2	1
Raomam	1	2	1	1	0	1	3	NA	0	1	0	0	1	0	0	2	0
Raomon	0	2	1	1	0	1 | 2	3	3	1	1	0 | 1	0	1	0	2	0	1
Raopet	0	1	1	2	0	1	1	4	0	1	0	0	0	0	0	1	1
RaospL	0	1	0	NA	1	1	4	4	0	1	2	0	0	0	2	2	2
RaospM	0	1 | 2	1	2	0	1	1 | 2	2	0	1	0 | 2	0	1	0	2	0	2
Raosub	0	2 | 3	1	1	0	1	2 | 3	3 | 4	0 | 1	1	0	0	1	0	1	0	2
Raosuu	0	2	1	2	0	1	2 | 3	4	0	1	0	0	0	0	2	2	2
Raoten	0	4 | 5	1	1	0	1	1 | 2	1 | 2	0	1 | 3	1	0	1	0	1	2	1
Raoyou	0	1	1	2	0	1	2 | 3	4	0	1	2 | 5	0	0	0	2	2	2

Symbols indicate: or (|), and (&), unknown (?), not applicable (NA).

Character No.[A]	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Anarup	0	0	1	1	0	2 | 3	1	0	NA	0	0	0	0	1	0	0	0
Anasub	0	0	0	0	1	2 | 3	1	0	NA	0	0	0	1	1	0	0	1
Anaker	0	0	1	1	0	2 | 3	1	1	1	0	0	0	1	1	0	0	0
Anatri	0	1	0	1	0	2 | 3	1	0	NA	1	0	0	1	1	0	0	0
Casacu	0	0	0	0	2	4	4	1	2	0	1	0	0	0 | 1	0	0	0 | 1
Caslon	0	0	0	0	2	2 | 3 | 4	2 | 4	1	1 | 2	1	0	0	0	0	0	1
Ewacat	0	0	1	1	1	2 | 3	1 | 2 | 3	0 | 1	1	0	0	0	1	0	0	0
Ewamer	0	2	1	1	0	2 | 3	1 | 3	0	NA	1	1	0	0	1	0	0	0
Ewapla	0	2	0	1	0	2 | 3	2 | 3	0	NA	0	0	0	0	1	0	0	0
Ewasin	0	0	1	2	0	1 | 2 | 3	1	1	1	1	1	0	0	0 | 1	0	0	1
Gnaaud	0	1	0	2	0	2 | 3	1 | 2 | 3	1	1	0	1	0	0	1	0	0	0
Gnainv	0	1	0	2	0	2 | 3	1	1	1	0	0	0	0	1	0	0	0
Gnamac	0	2	0	2	0	2 | 3	1 | 2 | 3	0	NA	0	0	0	0	1	0	0	0
Gnanit	0	2	0	2	0	2 | 3	1	0	NA	0	0	0	0	1	0	0	0
Gnatra	0	0	0	2	0	1 | 2 | 3	2 | 3	1	1	0	1	0	0	1	0	0	0
Haapul	0	2	0	1	0	1	1	0	NA	0	0	0	0	0	0	0	0	1
Haasin	0	2	0	0	0	2 | 3	2 | 3	0	NA	0	0	0	0	0	0	0	1
Helbel	0	0	1	1	1	1 | 2 | 3	1	0 | 1	1	0	0	0	0	0 | 1	0 | 1	0 | 1	0
Helcor	0	0	1	2	0	2 | 3	1	0	NA	0	0	1	0	0	0	0	1
Heldep	0	1	0	1	0	1 | 2 | 3	2 | 3	0	NA	0	1	1	0	0	0	0	1
Heldim	0	1	0	0	0	2 | 3	1	0	NA	0	0	1	0	1	0	0	1
Helfil	0	1	2	2	0	2 | 3	2 | 3	1	1	0	1	0	0	1	0	0 | 1	0
Helint	0	0	0	1	1	1 | 2 | 3	2 | 3	0	NA	0	0	0	0	0	0	0	1
Hellan	0	2	0	1	0	1 | 2	1	1	1 | 2	0	1	0	0	0	0 | 1	0	0	1
Helpar	0	2	0	0	0	1 | 2 | 3	1	0	NA	0	0	0	0	0	0	0	0	1
Leugra	0	0	1	2	0	1 | 2 | 3	1	0	NA	1	0	0	0	0 | 1	1	0 | 1	0 | 1
Leuleo	0	1	1	1	0	2 | 3	?	0	NA	0	0	0	0	0	0	0	1
Ozolep	0	2	0	1	1	2	2 | 3	1	2	0	0	0	0	0	0	0	1
Ozoobc	0	1	0	2	0	2	2 | 3	1	1 | 2	1	1	0	0	0 | 1	0	0	1
Ozorod	0	0	1	1	1	2	2	1	2	0	0	1	0	1	0	0	1
Pselut	0	2	1	2	0	1 | 2	1 | 2	1	1	0	0	0	0	1	0	0	0
Ptelaw	0	1	0	1	1	1 | 2 | 3	1 | 2 | 3	0	NA	0	0	0	0	1	0	0	0
Racgla	0	0	0	0	1	2 | 3	1	0	NA	0	1	0	0	1	0	1	0
Raoaus	0	1	1	1	1	2 | 3	1	0	NA	0	1	0	0	0	1	0	0
Raobry	0	1	2	1	0	2 | 3	1	0	NA	0	0	0	0	0	0	0	1
Raocin	0	2	0	1	0	2 | 3	1 | 2 | 3	0 | 1	1	1	0	0	1	0	0	0
Raoexi	1	2	1	1	0	2 | 3	1	0	NA	0	0	0	0	0	0	0	1
Raogla	0	2	0	2	0	2 | 3	1 | 2 | 3	1	1	0	1	0	0	0 | 1	0 | 1	0	0
Raogra	0	1	1	2	0	1 | 2 | 3	1	0	NA	0	0	0	0	1	0	0	0
Raohaa	0	2	0	0	0	1	1	0	NA	0	1	0	0	0	0	0	0	1
Raohec	0	1	2	2	0	1	1	0	NA	0	0	0	0	0	0	0 | 1	1
Raohoo	0	2	0	1	0	2 | 3	1 | 2 | 3	0	NA	0	0	0	0	1	0	1
Raomam	0	1	2	1	0	1 | 2 | 3	1	0	NA	0	0	0	0	0	0	0	1
Raomon	0	2	0	2	0	2 | 3	1	1	1	1	1	0	0	1	0	0	0
Raopet	0	0	1	1	0	2 | 3	1	0	NA	0	0	0	0	1	0	0	0
RaospL	0	1	0	0	1	2 | 3	1	0	NA	0	0	0	0	1	0	0	0 | 1
RaospM	0	0	0	2	0	1	1	0	NA	1	1	0	0	1	0	0	0
Raosub	0	2	0	2	0	1 | 2 | 3	1	0	NA	0	1	1	0	0	1	0	0
Raosuu	0	2	0	2	0	2 | 3	1 | 2 | 3	0	NA	0	0	0	0	0 | 1	0	0	0 | 1
Raoten	0	1	1	2	0	2 | 3	1	1	1	1	1	0	0	1	0 | 1	1	0 | 1
Raoyou	0	1	1	2	0	2 | 3	1	0	NA	0	0	0	0	0 | 1	0	0	1

Symbols indicate: or (|), and (&), unknown (?), not applicable (NA).

Character No.[A]	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Anarup	NA	5	3	1	5	0	1	0	2	0	0	1	0	0	0	0	0
Anasub	1	3	2	2	3	0	1	0	2	0	0	0	0	0	0	0	0
Anaker	NA	3	2	2	3	0	1	0	2	0	0	0	0	0	0	0	0
Anatri	NA	3	2	2	3	0	1	0	2	0	0	0	1	1	0	0	0
Casacu	1	3	2	1 | 2	3 | 7	1	2	0	1	0	0	0	2	1	1	0	0
Caslon	1	3	2	2	3 | 5	1	3	0 | 1	1	0	0	0	0	1	0	1	0
Ewacat	NA	3	2	1 | 2	3	0	1	3	1	0	0	0	0	0	0	0	0
Ewamer	NA	1	1	NA	1	0	1	5	1	2	3	0	0	0	0	0	0
Ewapla	NA	3	2	1 | 2	3	0	1	0	1	0 | 2	2	0	0	0	0	0	0
Ewasin	1	3	2	1 | 2	3	0	1	0	1	0	0	0	0	1	0	0	0
Gnaaud	NA	3	2	?	3	0	1	0	1	0	0	0	0	0	0	0	1
Gnainv	NA	3	2	1	3 | 5	0 | 1	3	0	1	0	0	0	1	1	1	0	0
Gnamac	NA	3	2	1 | 2	3	0	1	0	1	0	0	0	0	0	0	0	1
Gnanit	NA	3	2	1 | 2	3	0	1	3	1	0	0	0	0	0	0	0	0
Gnatra	NA	3	2	1	3	0	1	0	1	0	0	0	0	0	0	0	0
Haapul	1	3	2	1 | 2	3 | 5	0 | 1	3	0 | 1	1	0	0	1	0	0	1	1	0
Haasin	2	3	2	1 | 2	3 | 7	0 | 1	3	0	1	0	0	1	0	0	1	0	0
Helbel	NA	3	2	1	3	0	1	0	1	0	0	0	0	0	0	0	0
Helcor	2	1	1	NA	1	0	1	0 | 1	1	0	1	0	0	2	0	0	0
Heldep	1	1	1	NA	1	0	1	0	1	0	0	1	0	0	0	1	0
Heldim	2	1	1	NA	3	1	2	0	1	0	0	1	0	1	0	1	0
Helfil	NA	3	2	1 | 2	3	0	1	0 | 1	1	0 | 1	0	0	0	0	0	0	0
Helint	1	1	1	NA	1	0	1	0	1	0	1	0	0	1	0	0	0
Hellan	1	3	2	1 | 2	3	0	1	0	1	1	1	1	0	1	0	0	0
Helpar	1	1	1	NA	1	0	1	0	1	0	1	0	0	0	0	0	0
Leugra	1	3	2	1	3 | 5	0 | 1	3	0 | 3	1	0	0	0	0	0	0	0	0
Leuleo	1	3	2	2	3	0	1	0 | 3	1	0	1 & 2	0	0	0	0	0	0
Ozolep	1	1 | 2 | 3	1 | 2	1	1 | 2 | 3	0	1	2	1	1	0	1	0	1	0	0	0
Ozoobc	1	3	2	2	3 | 7	1	4	0 | 1	1	0	0	0	0	0	0	1	0
Ozorod	1	3	2	1 | 2	3 | 7	1	4	0	1	0	0	1	0	0	0	0	0
Pselut	NA	3	2	1	3 | 9	0 | 1	3	0	1	0	0	1	1	0	0	0	0
Ptelaw	NA	1	1	NA	1	0	1	0 | 1	1	0	0	0	0	0	0	0	0
Racgla	NA	3	2	1	3 | 7	0 | 1	?	0 | 1	1	0	0	1	0	0	0	0	0
Raoaus	1	1	1	NA	1	0	1	2	1	0	0	0	0	0	0	0	0
Raobry	1	1	1	NA	1	0	1	2	1	0	0 | 1	0	1	0	0	0	0
Raocin	NA	3	2	1 | 2	3	0	1	0 | 1	1	0	0	0	0	0	0	0	0
Raoexi	1	1	1	NA	1	0	1	0	1	0	0	0	0	0	1	0	0
Raogla	1	3	2	1 | 2	3	0	1	1	1	0	0	0	0	0	0	0	0
Raogra	NA	3	2	1	3	0	1	0	1	0	2	0	0	0	0	0	0
Raohaa	1	1	1	NA	1	0	1	2	1	0	0	0	0	0	0	0	0
Raohec	2	3	2	1	3	0	1	3	1	2	0	0	0	0	0	0	0
Raohoo	1	3	2	NA	3	0	1	2 | 3	1	0	0	0	0	0	0	0	0
Raomam	1	1	1	NA	1	0	1	2	1	0	0	0	0	0	0	0	0
Raomon	NA	3	2	1 | 2	3	0	1	1	1	0	0	0	0	0	0	0	0
Raopet	NA	3	2	1	3	0	1	1	1	0	0	0	0	0	0	0	0
RaospL	2	1	1	NA	1	0	1	4 | 5	1	0	0 | 2	0	0	0	0	0	0
RaospM	NA	1	1	NA	1	0	1	2 | 3	1	0	0	0	0	0	0	0	0
Raosub	NA	3	2	1	3	0	1	1	1	0	0	0	0	0	0	0	0
Raosuu	1	1	1	NA	1	0	1	3	1	2	2	0	0	0	0	0	0
Raoten	1	3	2	1	3	0	1	0	1	0	0	0	0	0	0	0	0
Raoyou	2	3	2	1 | 2	3	0	1	0	1	0	0	0	0	0	0	0	0

Symbols indicate: | (|), and (&), unknown (?), not applicable (NA).

APPENDIX 4: QUANTITATIVE STEM MEASUREMENTS

The quantitative measurements from the transverse stem sections for each specimen. The columns indicate the radius of the stem, cortex, phloem, xylem and pith (μm) in the mature stem sections for each species (columns three to seven), as measured along the maximum radius of stem in each specimen, and the maximum diameter (μm) of the vessels in each of the mature stem sections (last column).

Species	Collection No.	stem	cortex	phloem	xylem	pith	vessel
Anaphalis keriensis	CANU 37664	983	228	72	309.5	373.5	15.45
Anaphalis rupestris	JW 91135/2	1183	98	149	344.5	591.5	51.52
Anaphalis sugrigida	JW 94218	1538	185	151	754.5	447.5	34.59
Anaphalis trinervis	JW 94245	1407	357	63	229	758	29.9
Cassinia aculeata	JW 94115	967	41	196	199.5	530.5	31.73
Cassinia aculeata	JW 96103	1177	0	272	741	164	30.34
Cassinia longifolia	JW 94101	1994	0	235	969	790	49.48
Cassinia longifolia	JW 96102	1418	14	442	615	347	42.14
Ewartia catipes	JW 94081/1	538	170	68	150	150	17.07
Ewartia catipes	JW 94098/7	719	435	32	147	105	13.95
Ewartia meredithiae	JW 94040/1	469	278	61	44.5	85.5	13.98
Ewartia meredithiae	JW 94118/1	393	199	45	107.5	41.5	18.26
Ewartia planchonii	JW 94043/5	679	290	66	128	195	25.6
Ewartia planchonii	JW 96100	382	167	52	15	148	11.3
Ewartia sinclairii	CANU 37722	728	83	123	238	284	17.92
Ewartia sinclairii	CANU 37807	739	0	76	294	369	18.02
Gnaphalium audax	CANU 37550	780	237	83	166.5	293.5	15.75
Gnaphalium audax	CANU 37642	1078	335	69	280.5	393.5	18.29
Gnaphalium involucratum	CANU 37594	1046	184	129	174	559	28.58
Gnaphalium involucratum	JW 91139	1391	396	135	69.5	790.5	18.88
Gnaphalium mackayi	CANU 37674	543	252	29	112	150	18.02
Gnaphalium mackayi	CANU 37747	462	159	30	76	197	10.27
Gnaphalium nitidulum	CANU 37801	539	138	74	195.5	131.5	13.16
Gnaphalium nitidulum	JW 96045/2	420	173	51	92.5	103.5	12.44
Gnaphalium traversii	CANU 37534	671	296	103	67.5	204.5	16.06
Gnaphalium traversii	CANU 37715	570	270	60	61.5	178.5	10.61
Haastia pulvinaris	CANU 37598	957	206	211	338.5	201.5	29.46
Haastia pulvinaris	CANU 37774	1750	0	554	858.5	337.5	27.22
Haastia sinclairii	CANU 37796	896	309	62	206	319	23.52
Haastia sinclairii	CANU 37682	924	403	95	219.5	206.5	25.87

Species	Collection No.	stem	cortex	phloem	xylem	pith	vessel
Helichrysum bellidioides	CANU 37575	924	143	115	182	484	26.82
Helichrysum bellidioides	CANU 37796	1174	106	240	524.5	303.5	39.73
Helichrysum coralloides	CANU 37812	1226	190	122	598	316	22.34
Helichrysum coralloides	CANU 37717	1957	388	350	764.5	454.5	19.6
Helichrysum depressum	CANU 37535	1096	0	204	777	115	22.99
Helichrysum depressum	CANU 37618	1136	0	446	557	133	23.81
Helichrysum dimorphum	CANU 37706	803	93	128	324	258	29.97
Helichrysum filicaule	CANU 37579	555	99	78	77	301	23.76
Helichrysum filicaule	CANU 37638	507	87	38	85	297	17.49
Helichrysum intermedium	CANU 37573	1347	317	171	753.5	105.5	22.67
Helichrysum intermedium	CANU 37611	2201	276	132	1600.5	192.5	20.41
Helichrysum lanceolatum	CANU 37635	2916	30	434	1609.5	842.5	28.99
Helichrysum lanceolatum	CANU 37728	2012	0	304	1040	668	28.73
Helichrysum parvifolium	CANU 37599	1134	288	129	585.5	131.5	24.19
Helichrysum parvifolium	CANU 37763	1284	291	101	789	103	19.06
Leucogenes grandiceps	CANU 37557	1080	83	140	590	267	34.21
Leucogenes grandiceps	CANU 37751	647	116	45	207.5	278.5	13.77
Leucogenes leontopodium	JW 94225	2115	0	281	1487	347	20.72
Ozothamnus leptophyllus	CANU 37615	1721	294	213	1075	139	28.76
Ozothamnus leptophyllus	CANU 37546	1144	120	274	588	162	20.3
Ozothamnus leptophyllus	CANU 37788	3096	223	573	1905.5	394.5	30.32
Ozothamnus obcordatus	JW 94113	1412	26	279	899	208	14.86
Ozothamnus obcordatus	JW 96125	1462	0	253	860	349	30.19
Ozothamnus rodwayi	JW 94035	1586	187	275	709	415	30.03
Ozothamnus rodwayi	JW 94117	1694	141	306	804	443	28.61
Pseudognaphalium luteoalbum	CANU 37993	819	218	81	97.5	422.5	18.78
Pseudognaphalium luteoalbum	CANU 37560	1260	118	178	502	462	18.89
Pterygopappus lawrencei	JW 94077	347	167	39	97.5	43.5	11.13
Pterygopappus lawrencei	JW 94094	301	153	36	83	29	18.12
Rachelia glaria	CANU 35555	727	255	79	136	257	13.05
Raoulia australis	CANU 37538	683	0	193	391	99	25.99
Raoulia australis	CANU 37718	595	163	84	234	114	25.8
Raoulia bryoides	CANU 37616	1294	73	328	739	154	25.48
Raoulia bryoides	CANU 37773	557	102	84	189.5	181.5	11.42
Raoulia cinerea	JW 89091/1	680	326	63	129.5	161.5	21.54
Raoulia cinerea	CANU 37799	1135	406	115	318	296	12.66
Raoulia eximia	CANU 37582	1932	62	300	1395	175	30.42
Raoulia eximia	CANU 37703	3970	0	481	3404.5	84.5	37.35
Raoulia glabra	CANU 37576	721	0	167	426	128	29.17

Species	Collection No.	stem	cortex	phloem	xylem	pith	vessel
Raoulia glabra	CANU 37648	933	329	124	175.5	304.5	16.05
Raoulia grandiflora	CANU 37593	587	254	55	169	109	27.96
Raoulia grandiflora	CANU 37625	797	360	84	140	213	13.56
Raoulia haastii	CANU 37537	1728	0	331	1320.5	76.5	63.83
Raoulia haastii	CANU 37650	1425	59	299	998.5	68.5	38.43
Raoulia hectorii	CANU 37563	915	243	67	470	135	17.75
Raoulia hectorii	CANU 37696	683	163	69	333.5	117.5	18.13
Raoulia hookeri	CANU 37795	762	105	156	353.5	147.5	30.02
Raoulia hookeri	CANU 37630	1300	300	335	440.5	224.5	26.26
Raoulia mammillaris	CANU 37588	1474	0	362	996	116	24.42
Raoulia mammillaris	CANU 37701	1112	0	289	640.5	182.5	24.66
Raoulia monroi	CANU 37542	568	171	93	116.5	187.5	20.06
Raoulia monroi	CANU 37643	496	208	48	93.5	146.5	15.42
Raoulia petriensis	JW 91150/1	568	240	44	192	92	13.65
Raoulia sp. "L"	JW 94026	752	309	108	231.5	103.5	12.01
Raoulia sp. "L"	CANU 37740	312	174	12	73.5	52.5	9.54
Raoulia sp. "M"	CANU 37714	278	91	48	65.5	73.5	11.66
Raoulia sp. "M"	CANU 37595	386	123	62	91.5	109.5	17.02
Raoulia subsericea	CANU 37553	416	39	81	210	86	19.58
Raoulia subsericea	CANU 37608	957	118	175	483	181	32.89
Raoulia subulata	CANU 37769	566	58	83	198	227	16.37
Raoulia subulata	CANU 37805	1139	52	213	657	217	16.72
Raoulia tenuicaulis	CANU 37577	1253	115	225	688	225	66.96
Raoulia tenuicaulis	CANU 37619	641	69	106	381	85	42.78
Raoulia youngii	CANU 37687	958	271	99	442.5	145.5	13.91
Raoulia youngii	CANU 37699	601	143	76	265.5	116.5	13.98

APPENDIX 5: PHENETIC ALGORITHM

The issue of character weighting is not new in phenetics and the validity and methodology of character weighting have been the subject of much past debate (these will not be reviewed here, but see Stuessy (1990, pp. 70-71)). Many proponents suggested that phenetics should be objective and repeatable, thus character weighting should not be used. However, this argument was effectively countered by Mayr (1964) who stated

> "Indeed, there is doubt that pure non-weighting exists. Any choice of characters is already in itself a weighting process."

Indeed many of the steps in a taxonomic analysis, whether cladistic, phenetic or otherwise, are influenced by the unavoidable subjective decisions of the researcher (e.g. choice of study organisms/OTUs, character selection, character coding, choice of algorithm, and clustering or optimisation method) (Stuessy, 1990).

It is often of interest to compare the results obtained when the same data set is analysed using different methods (e.g. Parnell and Waldren, 1996; Faith, 1997), for example using cladistics and phenetics to analyse the same data matrix. However, differences in the results obtained from different methods of analysis may sometimes result from differences in character coding, rather than from different methodologies. It is therefore desirable, as far as possible, to submit the same data set to both analysis methods. A problem, however, arises when the data set contains OTUs that have some variable characters, for example when the OTUs represent the combination of two independent samples of the same species. While cladistic programs such as PAUP (Swofford, 1991) are able to deal with multistate characters as an uncertainty or polymorphism, in phenetics each character that contains multiple states for any OTU may be recoded into a series of binary characters (see example below). Recoding characters in this way introduces the problem of character weighting. For instance, by dividing a qualitative character with N states into N binary characters, the character has the weight of $\frac{N_{steps}}{N_{xters} + N_{steps} - 1}$, where N_{steps} is the number of steps in the character, and N_{xters} was the number of characters prior to the splitting of this character. Before splitting, the character had the weight $\frac{1}{N_{xters}}$. Thus, if a character in a data set originally containing a total of 100 characters was recoded into three binary characters the weighting of that character would increase from 0.01 to 0.029.

The aim of the coefficient presented below is therefore to allow all characters in a data set containing taxa with multiple character states to have equivalent weighting when **submitted** to phenetic and cladistic analyses.

Gower's coefficient and the 'stepped' coefficient

Gower's General Coefficient of similarity is a combination of three similarity coefficients (Gower, 1971), allowing it to be used with quantitative, qualitative and binary characters (see below). The similarity for any pair of OTUs (i, j) is given by

$$S_{ij}=\frac{\sum S_{ijk}}{\sum n_{ijk}}$$

where S_{ijk} is the similarity of OTU_i and OTU_j for character k, and n_{ijk} is the number of characters compared for OTU_i and OTU_j.

The similarity for quantitative characters, and also multistate ordered characters, is given by the range-standardised coefficient

$$S_{ijk} = 1 - \left(\frac{|X_{ik} - X_{jk}|}{R_k} \right) \text{ and } n_{ijk} = 1 \qquad (1)$$

where X_{ik} is the value of OTU_i for character k, and R_k is the range for character k.

For qualitative characters Gower's General Coefficient uses the simple matching coefficient of Sokal and Michener (1958)

$$S_{sm} = \frac{N_{sp} + N_{sn}}{N_{sp} + N_{sp} + N_u} \qquad (2)$$

where N_{sp} is the number of shared positive states (i.e. $X_{ijk} \in \{1,1\}$), N_{sn} the number of shared negative states (i.e. $X_{ijk} \in \{0,0\}$), and N_u the number of unshared states (i.e. $X_{ijk} \in \{0,1\}$). So that for any qualitative character k $n_{ijk} = 1$, the similarity $S_{ijk} = 1$ if the character states are shared, or $S_{ijk} = 0$ if the states are unshared.

Dichotomous characters are differentiated from two state qualitative, or alternate, characters in that a shared absence of a character is not regarded as a similarity using the dichotomous rule. In terms of similarity this means that two OTUs with the absent state (i.e. 0) for an alternate character will have a similarity of 1 for that character (i.e. $S_{ijk} = 1$, $n_{ijk} = 1$), whereas in the dichotomous rule this character would be ignored (i.e. $S_{ijk} = 0$, $n_{ijk} = 0$).

The simple matching coefficient is used for alternate character, whereas Jackard's coefficient of similarity is used for dichotomous characters

$$S_J = \frac{Nsp}{Nsp + Nu} \tag{3}$$

where *Nsp* and *Nu* are given above. Thus for any dichotomous character $S_{ijk} = 1$ and $n_{ijk} = 1$ if the states are positive and shared; $S_{ijk} = 0$ and $n_{ijk} = 1$ if the states are unshared, $S_{ijk} = 0$ and $n_{ijk} = 0$ if the states are negative and shared.

As indicated above, a problem associated with Gower's General Coefficient of similarity, is that it is unable to cope with taxa that possess variable characters. The simplest way of dealing with variable taxa is to recode that character into a series of binary or dichotomous characters, hereafter referred to as stepped binary or stepped dichotomous characters. For example, the hypothetical data set presented in Table A contains two characters (3 and 6) for which OTU d has multiple states. In table B these have been recoded into two stepped characters.

OTU \ Character	1	2	3	4	5	6	7
a	1	2	1	1	1	3	2
b	2	2	3	3	1	2	3
c	2	2	3	3	1	2	3
d	2	1	2/3	2	2	1/3	1

Table A: Hypothetical data set of seven qualitative characters for four taxa.

OTU \ Character	1	2	3.1	3.2	3.3	4	5	6.1	6.2	6.3	7
a	1	2	1	0	0	1	1	0	0	1	2
b	2	2	0	0	1	3	1	0	1	0	3
c	2	2	0	0	1	3	1	0	1	0	3
d	2	1	0	1	1	2	2	1	0	1	1

Table B: Data set from Table A, with characters 3 and 6 recoded as stepped characters.

A major problem with recoding a character is that it alters the weighting of the characters, effectively reducing the similarity between taxa, especially the least similar taxa. For the OTUs a, b and c, the similarities as coded in Table A are

$$S_{ab} = 2/7 = 0.2857$$

$$S_{ac} = 1/7 = 0.1429$$

$$S_{bc} = 3/7 = 0.4286,$$

However, after character recoding, the similarities become

$$S_{ab} = 4/11 = 0.3636$$

$$S_{ac} = 4/11 = 0.3636$$

$$S_{bc} = 4/11 = 0.3636$$

using only the simple matching coefficient (S_{sm}), and

$$S_{ab} = 2/8 = 0.25$$

$$S_{ac} = 1/8 = 0.125$$

$$S_{bc} = 3/8 = 0.375$$

using the simple matching coefficient (characters 1, 2, 4, 5, and 7) and Jackard's coefficient (characters 3 and 6). Clearly using only the qualitative rule is not meaningful for this data set, while using the combination of coefficients reduces the similarity between the three OTUs. (For other small data sets on which this was tried using only the simple matching coefficient tended to increase the similarity between less similar taxa, and decrease the similarity value between more similar taxa, although this result depended on the number of taxa, characters recoded, and the number of character states in those characters.) The decrease in similarity values will become increasingly large as more characters are required to be recoded into stepped characters.

This problem can be overcome by reweighting each stepped character so that it returns to its original value in the data set. This can be achieved using the following similarity coefficient for a stepped dichotomous character (k)

$$S_{ijk} = \frac{\sum N_{sp}}{\sum N_{sp} + \sum N_u} \qquad \text{and } n_{ijk} = 1, \tag{4}$$

where ΣN_{sp} is the number of shared positive states in all steps of character k and ΣN_u is the number of unshared states in all steps of character k, for cases where $(\Sigma N_{sp} + \Sigma N_u) > 0$. If $(\Sigma N_{sp} + \Sigma N_u) = 0$, both S_{ijk} and n_{ijk} are equal to 0.

Using this coefficient (4) and simple matching coefficient data set above (Table B), gives the similarities

$$S_{ab} = 2/7 = 0.2857$$

$$S_{ac} = 1/7 = 0.1429$$

$$S_{bc} = 3/7 = 0.4286,$$

thus giving the same values for S_{ab}, S_{ac}, and S_{bc} as found using the qualitative rule on Table A. Therefore, using the stepped coefficient, it is now possible to calculate similarities for OTU d.

$$S_{ad} = 0.5/7 = 0.0714$$

$$S_{bd} = 1.5/7 = 0.2143$$

$$S_{cd} = 1.5/7 = 0.2143$$

Conclusion: The addition of the stepped similarity coefficient (Equation 4)to the three already included in Gower's General Coefficient of similarity allows data containing taxa with variable character states to be submitted with equal weighting to cladistic and phenetic analyses. The phenetic analyses in this study were calculated using Gower's General Coefficient of similarity plus the stepped dichotomous coefficient.